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MICROWAVE HEATING OF FRUIT JUICES: KINETICS OF ENZYME INACTIVATION / MICROBIAL DESTRUCTION AND EVALUATION OF ENHANCED THERMAL EFFECTS

by

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MICROWAVE ENHANCED ENZYME / MICROBIAL DESTRUCTION AND KINETICS

DEDICATION

o o o

To my mother who loves me at my worst
To my motivator, and most of all, my best friend, Christian Azar

o o o

ABSTRACT

Conventional thermal kinetics of enzyme inactivation and microbial destruction in fruit juices were studied in the pasteurization temperature range (50 to 90°C). Pectin methylesterase (PME), as the most heat resistant enzyme, in orange juice and *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, as the most common spoilage yeast and bacteria, respectively, in apple juice used as indicators were subjected to heat treatment in a well-agitated water bath. Based on gathered time-temperature profiles, effective portions of the come-up (CUT) and come-down (CDT) times (lags) were determined for inclusion in kinetic data handling. The inactivation/destruction kinetics followed typical first-order rate of reactions. Two fractions of PME with different thermal resistances were observed. The D-values of PME in orange juice (pH 3.7) varied from 155 s at 60°C to 2.92 s at 90°C with a z-value of 17.7°C for the heat sensitive fraction and from 1240 s at 60°C to 109 s at 90°C with a z-value of 31.1°C for the heat resistant fraction. The D-values were influenced by pH and soluble solids concentration with lower thermal resistance at lower pH and higher soluble solids concentrations. The D-values of *S. cerevisiae* (ATCC 16664) in apple juice (pH 3.4) ranged from 58 s at 50°C to 1.9 s at 70°C with a z-value of 13.4°C, whereas that of *L. plantarum* (ATCC 14917) ranged from 52 s at 55°C to 1.2 s at 80°C with a z-value of 15.9°C. Kinetic data obtained were used for subsequent comparison with those obtained during microwave heating.

A continuous-flow microwave heating system was set up and evaluated for obtaining kinetic parameters under microwave heating conditions. The outlet temperature was characterized as a function of fluid flow rate, heating volume and initial temperature. The average residence time of the fluid was calculated by dividing the volume of heated sample in a helical coil inside the microwave oven by the volumetric flow rate. Based on the time-temperature profile of the fluid along the tube length during microwave heating, which was experimentally determined, the residence time was converted to effective microwave heating time. The system was tested for PME inactivation at 60°C under microwave heating and isothermal holding and found suitable for gathering data on inactivation kinetics.

Kinetics of enzyme inactivation and microbial destruction at various temperatures under continuous-flow microwave heating conditions were then evaluated using the technique established above. The rates of inactivation/destruction varied depending on temperature. Taking into consideration the effectiveness of the CUT and contributory thermal inactivation during the CDT, the D-values were found to vary from 38.5 s at 55°C to 1.32 s at 70°C (pH 3.7) for PME, 4.75 s at 52.5°C to 0.378 s at 60°C (pH 3.4) for *S. cerevisiae* (ATCC 16664) and 14.1 s at 57.5°C to 0.327 s at 65°C (pH 3.4) for *L. plantarum* (ATCC 14917). Microwave was thus found to be more superior to conventional thermal mode for enzyme inactivation / microbial destruction. Comparative studies of PME inactivation kinetics under batch-mode microwave heating conditions also confirmed an order of magnitude higher inactivation rate associated with microwave heating.

Some non-thermal microwave effects were hypothesized to exist and responsible for such differences between the two heating modes. Enzyme inactivation and microbial destruction were then studied further to evaluate the non-thermal effects. A continuous-flow microwave heating system was developed operating at full power while maintaining sample temperatures below 40°C by circulating a microwave-transparent liquid (kerosene) for immediate removal of heat produced in the juice during microwave exposure. A second set-up permitted a batch-mode microwave exposure of the test sample with a continuous mixing, again < 40°C, by submerging a cooling coil in the test solution. Results from these two set-ups showed insignificant inactivation/destruction thereby refuting the existence of non-thermal microwave effects under these conditions.

In order to explain and better characterize the faster rate of inactivation/destruction associated with microwave heating conditions observed in kinetic studies, additional tests were carried out using the second set-up described above, but without the cooling heat exchanger. The temperature of samples of different sizes were allowed to progressively increase under carefully controlled conditions. Inactivation of PME in orange juice (pH 3.7) and destruction of *S. cerevisiae* in apple juice (pH 3.4) were again used as parameters. The results once again clearly demonstrated superior inactivation/destruction effects under microwave heating which increased with temperature and decreased with sample size. The phrase "enhanced thermal effects" was, therefore, used to describe this

behaviour which was apparent only at temperatures beyond 50°C. The enhanced thermal effects were characterized in terms of a microwave enhancement ratio (MER), defined as ratio of microwave to thermal inactivation of enzyme or destruction of microorganism (on logarithmic scale), with values greater than 1.0 indicating positive enhanced effects. The MER values were found to vary with sample size and temperature, and values as high as 10 to 20 were observed for small samples (< 100 g). Microwave heating conditions (sample size, temperature and microwave power capacity) which would permit enhanced microwave effects were identified.

RÉSUMÉ

La cinétique conventionnelle d'inactivation enzymatique et de destruction des microorganismes dans les jus de fruits ont été étudiées à des températures de pasteurisation variant de 50 à 90°C. C'est la pectine méthylestérase (PME) qui a été choisie parce qu'étant l'enzyme la plus résistante à la chaleur, dans le jus d'orange. Par ailleurs, deux microorganismes reponsables de la dégradation, soit *Saccharomyces cerevisiae* (une levure) et *Lactobacilus plantarum* (une bactérie), ont été sélectionnés pour l'évalution de leur cinétique d'inactivation dans le jus de pomme. De petits échantillons ont été soumis à des traitements de chaleur variés dans un bain thermostaté bien agité. En se basant sur les profils de temps-température obtenus, les portions effectives de temps durant la mise en température et le refroidissement ont été déterminées pour la manipulation des données par intégration numérique des effets thermiques desquels on a calculé le taux d'inactivation. On a observé que les cinétiques d'inactivation/destruction suivent une réaction typique du premier ordre. Deux fractions de PME ayant des résistances thermiques différentes ont été identifées. La valeur de D de la PME dans le jus d'orange (pH 3.7) a varié de 155 s à 60°C à 109 s à 90°C avec une valeur de z de 17.7°C pour la fraction sensible à la chaleur et de 1240 s à 60°C à 109 s à 90°C avec une valeur de z de 31.1°C pour la fraction résistante. On a trouvé que le qu'un pH bas et qu'une quantité de solides solubles plus élevé ont engendré une résistance moindre. Les valeur s de D de *S. cerevisiae* (ATCC 16664) dans le jus de pomme (pH 3.4) on varié de 58 s à 50°C à 109 s à 70°C avec une valeur de z de 13.4°C alors que celles du *L. plantarum* (ATCC 14917) se situaient entre 52 s à 55°C à 1.2 s à 80°C avec une valeur de z de 15.9°C. Les données de cinétique obtenu ont été utilisées pour comparison aux valeurs obtenues durant le chauffage microonde.

Un système à débit continu de chauffage microonde a été mis en place et évalué pour obtenir les paramètres cinétiques. La température à la sortie du système a été caractérisée comme une fonction du débit de jus, du volume de chauffage et de la température initiale. Le temps de résidence du fluide a été calculé en divisant le volume de l'échantillon, chauffé dans un serpentin hélicoïdal placé à l'intérieur d'un four

microonde conventionnel, par le débit volumétrique. En se basant sur les profils de temps-température de fluide sur toute la longueur de tube durant le chauffage microonde, déterminés expérimentalement, le temps de résidence a été converti en temps de chauffage microonde effectif. Le système a été vérifié expérimentalement pour la cinétique d'inactivation de la PME à 60°C durant le chauffage microonde et une retenue isothermique. Le système s'est avéré souhaitable pour la collecte de données sur la cinétique d'inactivation.

Ainsi, la cinétique d'inactivation enzymatique et la destruction des microorganismes à différentes températures ont été évaluées dans ces conditions. Les taux d'inactivation/destruction ont varié dépendamment de la température. En considérant la partie effective du temps de mise en température et la contribution au cours du refroidissement, on a trouvé que les valeurs de D ont varié de 38.5 s à 55°C à 1.32 s à 70°C (pH 3.7) pour la PME et 4.75 s à 52.5°C à 0.378 s à 60°C (pH 3.4) pour *S. cerevisiae* (ATCC 16664) et 14.1 s à 57.5°C à 0.327 s à 65°C (pH 3.4) pour *L. plantarum* (ATCC 14917). Le chauffage microonde s'est avéré plus efficace ou supérieur au chauffage conventionnel pour l'inactivation d'enzymes et la destruction de microorganismes. L'étude comparative de la cinétique d'inactivation de la PME a démontré la supériorité, par un ordre de grandeur du taux d'inactivation, du chauffage microonde statique comparativement au chauffage conventionel.

On a suggéré l'existence d'effets non-thermiques qui pourraient être responsables des différences entre ces deux modes de chauffage. L'inactivation enzymatique et la destruction microbienne on donc été étudiés pour mieux comprendre les effets non-thermiques. Un système microonde à débit continu fut développé. Ce système fonctionnait à puissance maximale tout en maintenant la température des échantillons en-dessous de 40°C par recirculation d'un liquide transparent aux microondes (kérosène) pour enlèvement immédiat de la chaleur produite dans les échatillons durant l'exposition aux microondes. Dans un deuxième système de chauffage microondes statique, a agitation continue, l'échantillon était maintenu à basse température (<40°C) en y immergeant un serpentin refroisissant. A cause d'une inactivation/destruction non significative, les résultats obtenus de ces deux systèmes ont contredit l'existence des effets non-thermiques

dus aux microondes.

Pour expliquer et caractériser les taux d'inactivation/destruction plus rapides associés aux conditions de chauffage microonde, des tests supplémentaires ont été réalisés en utilisant le système statique sans l'utilisation du système d'échange de chaleur. Les températures des échantillons de différentes grosseurs ont été progressivement augmentées dans des conditions contrôlées. L'inactivation de la PME dans le jus d'orange (pH 3.7) et la destruction de *S. cerevisiae* (ATCC 16664) dans le jus de pomme ont été utilisées comme paramètres d'évaluation. Les résultats indiquent de nouveau et clairement une meilleure inactivation/destruction avec le chauffage microonde lorsque la température augmente ou que la grosseur de l'échantillon diminue. On a donc conclu que les effets thermiques sont rehaussés d'où la phrase "enhanced thermal effects" pour décrire et explique ce comportement qui n'est apparent qu'à des températures au-delà de 50°C. Les effets thermiques rehaussés ont été caratérisés en fonction du terme ratio rehaussement des microondes (RRM), défini comme le ratio d'inactivation microonde/thermique de l'enzyme ou de la destruction des microorganismes (sur une échelle logarithmique), avec des valeurs plus grandes que 1.0 indiquant des effets rahausseurs positifs. On a trouvé que les valeurs RRM étaient dépendantes de la température et de la grosseur des échantillons, où des valeurs aussi élevées que 10 ou 20 ont été observées pour de petits échantillons (<100 g). Des conditions de chauffage au microonde (grosseur des échantillons, température et puissance du microonde) qui peuvent engendrer des effets rehausseurs des microondes ont été identifiées.

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MANUSCRIPTS PUBLISHED/PREPARED BASED ON THIS THESIS RESEARCH

1. **Tajchakavit, S.** and Ramaswamy, H. S. 1995. Continuous-flow microwave heating of orange juice: Evidence of nonthermal effects. J. Microwave Power. 30(3): 141-148.

2. **Tajchakavit, S.** and Ramaswamy, H. S. 1997. Thermal vs. microwave inactivation kinetics of pectin methylesterase in orange juice under batch mode heating conditions. Lebensm. - Wiss. u. Technol. 30:85-93.

3. **Tajchakavit, S.** and Ramaswamy, H. S. 1997. Continuous-flow microwave inactivation kinetics of pectin methyl esterase in orange juice. J. Food Proc. Presv. *(In press)*

4. **Tajchakavit, S.**, Ramaswamy, H. S. and Fustier, P. 1997. Microwave and thermal destruction kinetics of spoilage microorganisms in apple juice. *(In preparation)*

5. Ramaswamy, H. S. and **Tajchakavit, S.** 1997. Study of non-thermal inactivation of enzyme and destruction of microorganism during microwave heating at sublethal temperatures. *(In preparation)*

6. Ramaswamy, H. S. and **Tajchakavit, S.** 1997. Enhanced thermal effects of microwave heating on enzyme inactivation and microbial destruction at elevated temperatures. *(In preparation)*

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8. **Tajchakavit, S.** and Ramaswamy, H. S. 1997. Enhanced thermal effects of microwave heating on enzyme inactivation. CoFE, Los Angeles, CA, November 19-21 *(to be presented)*.

7. **Tajchakavit, S.**, Ramaswamy, H. S. 1997. Microwave inactivation of pectin methylesterase in orange juice: Evidence of enhanced thermal effects. CIFST, Montreal, PQ, September 21-24.

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NOMENCLATURE

A	Mean residual enzyme activiy, unit/mL
C	Concentration of any component of interest
C_p	Specific heat, kJ/kg °C
D, D_1, D_2	Decimal reduction time, D at T_1, D at T_2, s
E_a	Activation energy, kJ/mole
F	Process lethality, s
j	Complex constant
k, k_1, k_2	Reaction rate constant, k at T_1 , k at T_2, s^{-1}
L	Lethal rate
m	Mass of test sample, kg
n	Order of reaction
P	Power, W
Q	Quantity of heat, kJ
R	Universal gas constant, kJ/mole K, Heating rate, °C/s
S	Frequency factor
t	Time, s
T	Temperature, °C
tan δ	Dielectric loss tangent
t_c	Effective cooling time, s
t_e	Effective heating time, s
t_{eth}	Equivalent thermal time, s
t_L	Microbial lag phase, s
t_M	Mass normalized microwave heating time, s/kg
z	Temperature sensitivity indicator, °C

Greek Symbols

ε*	Relative complex permittivity
ε'	Relative dielectric constant
ε"	Relative dielectric loss
ρ	Density of fluid, kg/m^3

Subscripts

1, 2	Refer to two levels with respect to temperature
f	Final condition
i	Initial condition
o	Outlet
ref	Reference

Abbreviations

CDT	Come-down Time
CFU	Colony Forming Unit
CUT	Come-up Time
FCC	Federal Communications Commission, USA
HTST	High Temperature Short Time
IFT	Institute of Food Technologists
ISM	Industrial, Scientific and Medical
MER	Microwave Enhancement Ratio
MW	Microwave
PDA	Potato Dextrose Agar
PME	Pectin Methylesterase
RF	Radio Frequency
TDT	Thermal Death Time

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1. A methodology was developed for including lethal contributions during the lag (come-up and come-down) periods and employed for heating involving both isothermal and non-isothermal heating conditions. An iteration method and a developed computer program allowed to obtain more precise kinetics data.

2. Thermal kinetics for inactivation of PME in orange juice and destruction of major spoilage yeast and bacteria in apple juice at temperatures appropriate for pasteurization were evaluated with the consideration given to accommodate the effective portion of come-up and come-down periods.

3. The feasibility of employing microwave heating as an alternative pasteurization method for fruit juices was evaluated. This was the first study comparing lag corrected kinetic data from microwave and thermal inactivation under continuous and batch mode heating conditions.

4. This was the first time a clear superiority of microwave heating over conventional heating was confined using a full set of kinetic data.

5. Different designs of microwave heating units which allowed the juice to fully expose to microwaves at low temperature for evaluation of microwave effects were evaluated. The non-existence of non-thermal effects of microwave heating was confirmed with both enzyme inactivation and microbial destruction when carried out at low temperatures.

6. The observed superiority of inactivation/destruction kinetics under microwave heating conditions was demonstrated to be occurring only at elevated temperatures. A concept of microwave enhancement ratio was developed to explain these differences.

CHAPTER I

INTRODUCTION

Thermal processing has been a widely known and effective method for food preservation for almost two centuries. Its primary objective is to extend shelf life of foods with assurance of safety by destroying food pathogens and by reducing or preventing food deterioration arising from enzyme activity and microbial spoilage as well as chemical reactions. The heat treatment however affects quality attributes of food products especially thermolabile nutrients and organoleptic qualities. Optimization of thermal processing for safety and quality has been the main theme for producing better quality foods. Several techniques including microwave heating, ohmic heating, and other non-thermal processing technologies such as high pressure processing, electrical pulse treatment, light pulses, ultrafiltration, irradiation, addition of preservatives or their combinations have been investigated as alternatives to conventional thermal processing (Mertens and Knorr, 1992). Since the development of these advanced techniques alter traditional products and processing regimes, increased emphasis, and special attention is required to confirm enzyme inactivation and microbial destruction.

Microwave processing is one of the processing techniques that offers good potential for high quality and reduced process times as compared with conventional processing techniques. Microwave ovens have gained widespread acceptance in home application for reheating and preparation of foods, and microwave processing has recently gained better acceptance as an effective method for food preservation in several processes such as cooking, drying, pasteurization and sterilization (Decareau, 1985). It has been claimed to offer several distinct advantages over conventional thermal techniques such as (1) improved retention of thermolabile constituents in the fluids such as milk and fruit juices due to the possibility of employing HTST processing conditions (Mudgett, 1986), (2) reduced process times due to rapid heating and (3) improved energy efficiency. In addition, the less obvious, but equally important, consideration is that it is environmentally friendly. The process thus has gained ground in the field of production.

Microwave heating also shows promise for liquid foods in situations where fouling of heat exchanger surfaces (plate/tubular) may cause problems. Fouling is a major problem resulting as deposits when food components are exposed to high temperature surfaces in continuous high-temperature short-time (HTST) pasteurization processes. These deposits on the inner surface of the pipes not only reduce the rate of heat transfer but also lead to off-flavor development and loss of nutritional attributes. Since microwave energy causes heat to be generated internally within the liquid as the wave passes through the microwave-transparent tubings or heating coils, the surface and surroundings do not need to be maintained at higher temperatures. Hence, the fouling problem could potentially be completely eliminated. Microwaves thus provide significant advantage over conventional thermal processing.

Several industrial microwave systems have begun operation in the U.S. (Schiffmann, 1992) and in Europe (Ohlsson, 1991). Successes reported with tempering of frozen meat and pasteurization of packed bread and pasta products gave considerable impetus to the concept which has now been extended to several other applications including drying of granular materials and cereals, vacuum drying of fruit juice concentrates, cooking of meat products and pasteurization and sterilization of prepared foods resulting in over 100 industrial systems mainly operated in Europe (Ohlsson, 1991). Although there has been an increasing demand for microwave processing of food, the growth of industrial microwave processing has not been widely apparent, a reason for which has been reported to be lack of information on product safety and quality (Mudgett, 1986).

Considerable research has been undertaken involving the use of microwaves for pasteurization of liquid foods especially milk (Hamid *et al.*, 1969; Jaynes, 1975; Chiu *et al.*, 1984; Merin and Rosenthal, 1984; Knutson *et al.*, 1988; Kudra *et al.*, 1991). However, the microwave pasteurization of fruit juices/beverages requiring enzyme inactivation and microbial destruction has not been commonly studied. Of the few studies carried out (Copson, 1954; Foley, 1985; Nikdel and MacKellar, 1992; Nikdel *et al.*, 1993; Abd El-Al *et al.*, 1994), very little information is available on kinetics of enzyme inactivation and microbial destruction. In addition, a major deficiency in many studies has been the

absence of effectiveness consideration during come-up and come-down (lag) periods.

The fruit juice processing industry is one of the world's major agro-based businesses. Currently, the market for fresh fruit juices in North America and other countries is getting rapidly expanded. In Canada alone, the market for refrigerated fruit juices, especially orange and apple juices, is valued at over $190 million dollars. Furthermore, as reported in 1996, 85 % of the households buy juices which constitutes steady 5 % annual increase over the past 6 years (Anon, 1997). Orange juice is the dominant juice traded worldwide followed by apple juice. Pasteurized fruit juices are high-priced premium quality products, but with a limited shelf life even at low refrigerated storage and distribution temperature. In order to establish a microwave pasteurization process, one needs to understand the mechanisms of action of the microwave process on food and food components such as enzymes and microorganisms. Presently, available data on kinetic parameters related enzyme inactivation or microbial destruction are limited.

It is believed that microwaves inactivate enzymes and kill microbes mostly by conventional thermal mechanisms. However, it is also generally recognised that the dielectric heating by microwaves affects biological systems by some other mechanisms. Investigations carried out over the decades are in abundance, however, there have been contradicting reports as to the existence or non-existence of non-thermal effects due to microwave heating. Several possible theories have been v proposed for the existence of non-thermal effects (Olsen, 1969; Rosen, 1972). Literature has implied such non-thermal effects and suggested application of microwaves for food preservation at low temperature. Several studies have refuted lethal effects of microwaves other than those contributed by heat. In reviewing the literature, several reasons have been found to preclude those studies, the main ones being doubts in temperature exposure of samples and comparison of microwave and conventional heating at unequivalent time-temperatures. Fung and Cunningham (1980) stated the need for technology that would allow comparisons of equivalent time-temperature treatments of microwave and conventional thermal heating. The IFT Expert Panel on Food Safety and Nutrition (Mudgett, 1989) stated that "... it is not possible to prove or disprove such claims, since the internal temperature of a bacterial

cell can not be measured." It is likely that some difficulties were encountered in this type of investigations, an improved experimental design, control and analysis of data can help to resolve the controversy.

The following are the objectives of this research :

1. To establish thermal kinetics of PME inactivation in orange juice and destruction of spoilage microorganisms, namely, *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in apple juice, as influenced by conventional heating with special consideration to come-up and come-down time effectiveness.
2. To evaluate heating characteristics of liquids in a continuous-flow microwave heating / isothermal holding system and to establish procedures for microwave kinetic studies.
3. To evaluate inactivation kinetics of PME in orange juice and destruction kinetics of *S. cerevisiae* and *L. plantarum* in apple juice during continuous-flow non-isothermal microwave heating, and compare them with their conventional thermal counterparts.
4. To confirm microwave inactivation kinetic behavior of PME in orange juice using batch-mode microwave heating conditions.
5. To evaluate the "*non-thermal*" and "*enhanced thermal*" effects of microwave heating with respect to PME inactivation in orange juice and destruction of *S. cerevisiae* in apple juice.

The present investigation is intended to provide a basis for the microwave pasteurization concept and process possibilities for fruit juices. In addition, considerable emphasis was laid on the clarification of the inconclusive issue on the existence of non-thermal microwave effects. Finally, it is hoped that this research will foster further development of the industrial microwave pasteurization process.

CHAPTER II

LITERATURE REVIEW

A general review of the historical development, how microwaves heat and interact with food and food components, its advantages and limitations, and technological applications in food processing are presented in this chapter.

Historical background/ retrospect

While Nicholas Appert, the pioneer of thermal processing, was still alive and thriving, Michael Faraday found the electromagnetic field in 1832. Microwave is the name given to electromagnetic waves arising as radiation from an electrical distribution across a broad spectrum of frequencies ranging between 300 MHz and 300 GHz. However, not until 1873, when Louise Pasteur had introduced the concept of pasteurization, James Clark Maxwell predicted the existence and behavior of these high frequency radio waves based on Faraday's hypothesis which was experimentally verified in 1885 by Heinrich Hertz in Germany. Jacques Arsène d'Arsonval also noted heat generation as the effect of high frequencies and later in 1895 introduced the first heat therapy unit referred to as d'Arsonvalization or diathermy. The magnetron, which is a microwave generator, was first developed in 1940 at the University of Birmingham in England. A prototype was then brought to the United States and, consequently, the microwave radar was developed. Radar (*ra*dio *d*etection *a*nd *r*anging) heating is the original term used for microwave heating. Among the first to recognise the potential of microwave applications in food processing were Proctor and Goldblith in the early 1940s. The advent of microwave ovens were reported just prior to World War II. Most work during that period were focused on manufacturing designs and communications for military purposes. Other developments including industrial microwave processing took place after World War II.

Early on, the Ratheon company was recognized as the most active group in microwave heating development and it developed and patented "Radarange" as the trademark for the microwave oven line. In 1947, a Radarange microwave oven was

introduced for cooking and food processing in restaurants and catering. Several potential uses of microwaves including freeze-drying, thawing of frozen foods, blanching of vegetables and roasting of coffee were initially developed at low frequencies during 1940s and 1950s.

Not until the mid-1940s, were microwaves at 2450 MHz explored. Several applications including blanching of vegetables, coffee roasting and cooking, and baking for better vitamin retention were reported. In addition, the first microwave freeze-dryer was introduced (Decareau and Peterson, 1986). In the early 1960s, industrial microwave processing began on a commercial scale. The first continuous microwave process with high power generating tubes was introduced during this period. Consequently, the Cryodry Corporation introduced the first conveyorized microwave system operating at 915 MHz and 25 kW. Simultaneously, Litton Industries developed a similar system at 2450 MHz and 2.5 kW (Decareau, 1985). The combination system using microwave and saturated steam with magnetron operating at 2450 MHz and 130 kW was introduced in 1966 for poultry processing. Among the successful industrial applications during that period was the microwave unit for finish drying of potato chips which was well accepted in the United States and in Europe (Decareau, 1985). Several other early studies were considered proprietary. While the popularity of the home microwave oven as a consumer appliance began in 1970s, commercial microwave systems for tempering and drying also hit their stride in the middle of the 1970s.

Principles of microwave heating

Microwave heating is also termed "radio frequency" or "electronic heating" which is related not only to the dielectric properties of dielectric materials such as foods, but also to electrical transmission properties (Decareau, 1985). The electromagnetic spectrum is illustrated in Figure 2.1. Due to the overlap of microwave frequencies with the radio frequencies used for telecommunication purposes, only certain frequencies are allocated by the Federal Communications Commission (US FCC) for Industrial, Scientific and Medical use (ISM). The most common frequencies used within North America are 915 MHz and 2450 MHz (Decareau, 1985). The ability of microwaves to penetrate a food

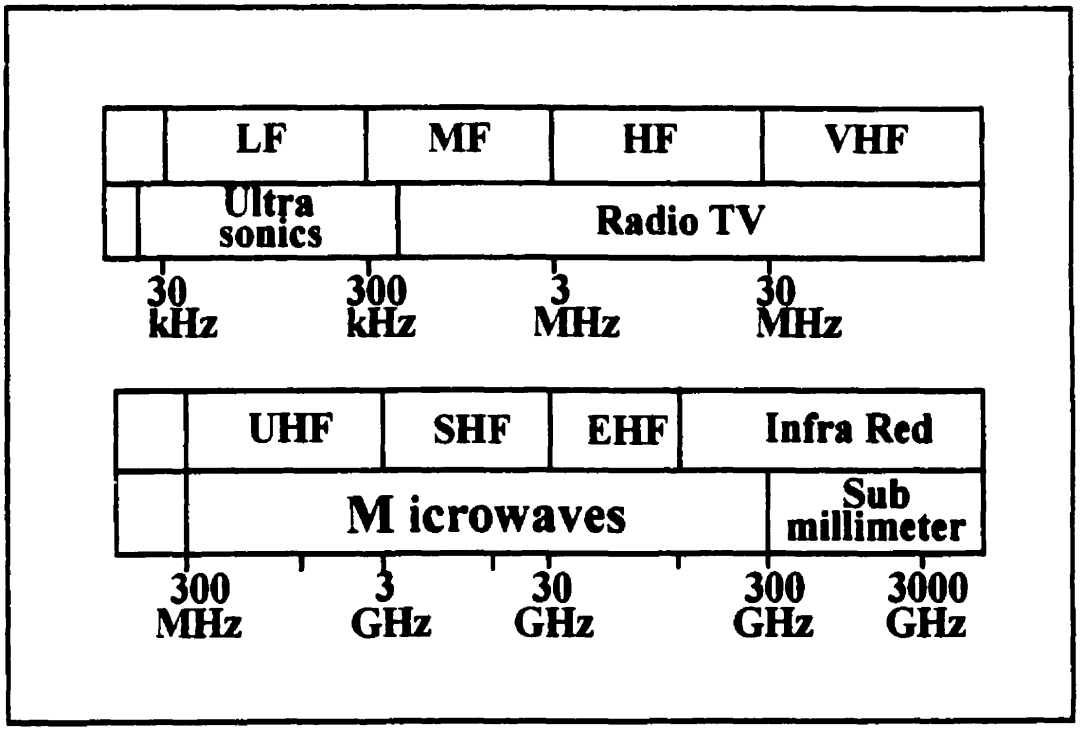

Figure 2.1 Electromagnetic spectrum (Source: Decareau and Peterson, 1986).

product causing rapid heating has attracted interest towards the exploitation of microwave technology. Microwave penetration depth is generally much deeper than that of conventional heating. The penetration depth decreases with an increase in frequency. Microwaves with a frequency of 915 MHz, therefore, have higher penetration than microwaves of 2450 MHz. The penetration depths range from 8 to 22 cm for 915 MHz and 3 to 8 cm for 2450 MHz depending on moisture content (Decareau, 1985).

Unit components of a microwave oven

The basic components of a microwave oven are well described by Decareau and Peterson (1986). In general, it consists of several components: power supply, power tube, transmission section, field stirrer, cavity and door. The power supply converts line voltage to several thousand DC voltage necessary to operate the power tube or magnetron. The magnetron is a microwave power generator which is commonly used in microwave ovens and microwave processing systems. The generated microwave energy is transferred from the vacuum power tube to the oven by means of a waveguide. In a microwave oven, the waveguide is only a few centimeters long whereas it could be up to 30 m or longer in an industrial system. Energy distribution in the oven is accomplished by employing a field stirrer which makes changes in field distribution in a confined space such as the oven cavity. The last component is the microwave oven door which provides access to the oven, and is specially designed with interlocking features to turn off the magnetron at the slightest opening of the oven door, thereby preventing leakage of microwave energy. The metal wall of the microwave oven reflects the microwaves, bouncing them back and forth until they get absorbed in to foods being heated.

Heating mechanisms

Heat is generated by the interaction of microwave with the materials being heated. Water, which is prevalent in most foods and which has a non-uniform charge distribution on the molecule, plays an important role in the microwave heating of foods. Water molecules attempt to align themselves (Figure 2.2) with the oscillating electric field and flip-flop several million times per second. As a result, heat is instantaneously generated

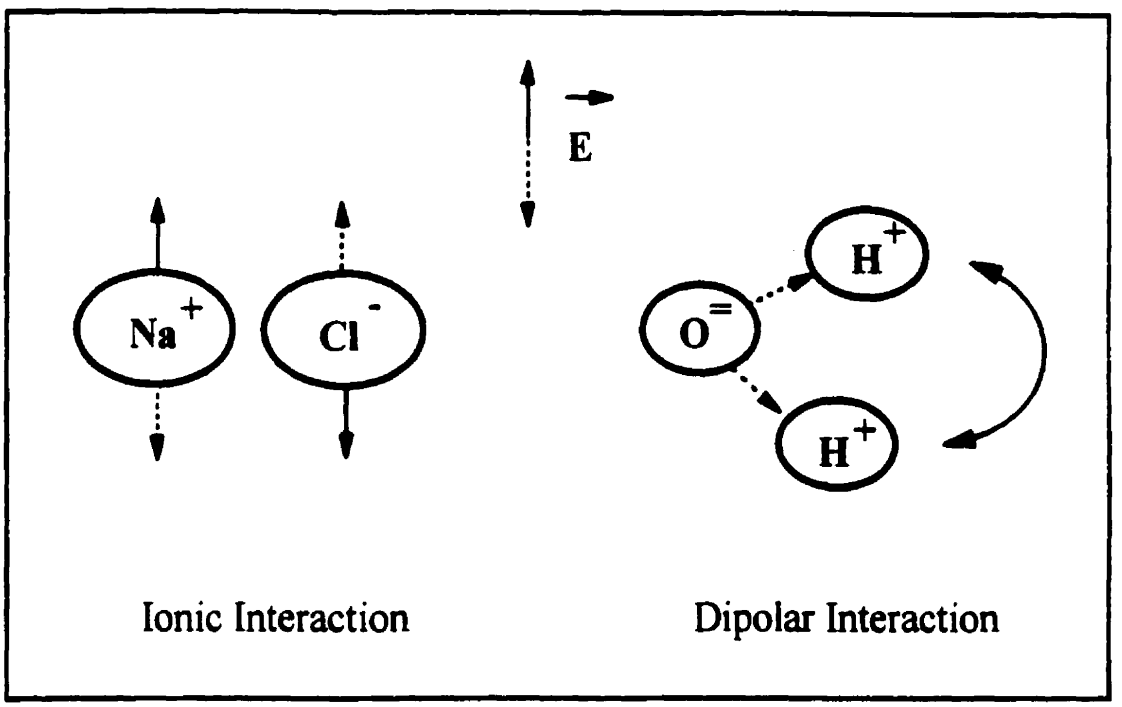

Figure 2.2 Microwave heating mechanisms (Source: Buffler, 1993).

due to internal molecular friction and is dissipated throughout the food material. Other constituents such as salts also contribute to heat generation by either friction or high speed electrophoretic migration in the electric field. Microwave interaction with the polar molecules also results in the rotation of molecules in the direction of the oscillating field and, in turn, collisions with other polar molecules occur thus causing heat generation. The interaction with water molecule, sodium ion, and chloride ion are illustrated in Figure 2.2. These interactions are also affected by the state of the constituents, whether they are bound or free, e.g., bound ions have much lower microwave absorptivities (Decareau and Peterson, 1986; Ramaswamy and van de Voort, 1990).

Parameters affecting microwave heating

The microwave heating of food materials is affected by a number of properties of microwaves and materials being heated (Schiffmann, 1986). The frequency and power of the microwaves are properties of the microwave system which contribute to the microwave heating characteristics. In addition, other factors related to the materials being heated also can influence the heating process.

Dielectric properties

The dielectric properties of food materials determine their ability to store and dissipate electrical energy from an electromagnetic field. The term "complex permittivity (ε^*)" governs the microwave absorptivity of food materials. The other two important components: the dielectric constant (ε') and the dielectric loss (ε'') can be related in the following equation (von Hippel, 1954):

$$\varepsilon^* = \varepsilon' - j\varepsilon'' \qquad (2.1)$$

where ε^* = relative complex permittivity, ε' = relative dielectric constant, ε'' = relative dielectric loss and j = constant.

The dielectric constant (ε') is a measure of the ability of the material to store microwave energy, while the dielectric loss (ε'') is a measure of the ability of the material to dissipate this energy as heat. The microwave absorptivity can also be expressed as a

dielectric loss tangent (tan δ), which is a ratio of the dielectric loss to the dielectric constant.

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \qquad (2.2)$$

Generally, the higher the value of these terms, the better the material will absorb microwave energy. These dielectric properties are considerably affected by several factors including the composition of foods (fat, protein, water, salt and solid content). In addition, temperature directly influences the dielectric properties particularly during a change of physical state. A large difference between the dielectric properties of ice (ε' = 3.2-3.3) and water (ε' = 78) is an example of how the physical state of foods affects their dielectric properties.

Initial product temperature

Absorption of microwave energy results in a certain temperature rise in the product. Hence, the heating time and the final product temperature will depend on the initial temperature of the product. Other factors of the food are also temperature dependent including dielectric and thermal properties.

Physical geometries

As summarized by Schiffmann (1986), the size and shape of food materials being heated play an important role in the distribution of heat within the food product. The closer the size is to the wavelength, the higher will be the center temperature. Smaller particulates require less heat than larger ones. In addition, the more regular the shape, the more uniform will be the heat distribution obtained within the product. A higher surface to volume ratio results in more rapid heating (Giese, 1992). Therefore, the heating rate for a sphere will be different from that for a cylinder with the same volume. The relationship between load geometry, load orientation and oven cavity parameters such as cavity size and geometry, however, are yet to be established (Buffler, 1993).

Thermal properties

The thermal properties of materials being heated such as the heat capacity and thermal conductivity strongly affect the product heating pattern. The heat capacity indicates the amount of energy required to raise the temperature of a unit mass of the food by a degree. Thus, fat which has lower heat capacity than water heats up faster than water. Since heat generated by microwaves is subsequently dissipated throughout the foods by conduction, the thermal conductivity also plays into a major role.

Density and consistency

The density of foods also determines how foods heat after microwave energy has been absorbed. A slower heating rate would obviously be observed in denser or more viscous foods (Schiffmann, 1986).

Advantages and limitations of microwave processing

The advantages of microwave processing over conventional thermal processing are obvious and have been summarized in the literature (Decareau, 1985; Mudgett, 1989; Giese, 1992). Food generally consists of more than 80% water thus heating up rapidly during microwave heating. Since the dielectric properties of water are high, this greatly accelerates the heating process resulting in significantly shorter process times as compared with conventional heating. As a result of the shorter process times, retention of thermolabile nutrients is improved. For viscous liquid foods, scraped surface heat exchangers have been developed to minimize fouling problems by continuously sweeping the hot surfaces with scraper blades. Microwave heating could also provide an alternate convenience by which surface fouling could be avoided since lower temperature gradients from the surface to the center can be expected thus rendering the tube surface cooler. Microwaves offer particularly more of an advantage to foods that are poorer in conductivity. In drying, although there is less amount of water, the available water selectively absorbs microwave energy and rapidly diffuses water vapor to the surface.

In addition to a number of advantages to the final products, the microwave processing offers convenience and benefit in the processing aspect. Energy consumption

is considerably reduced since microwave heating occurs only within the food not the surroundings and heat is produced only where it is needed. An increased processing rate and advances in designing microwave equipment have also lead to possible compact microwave equipments. Microwave processing also offers a flexibility to start-up and shut-down instantaneously and since the system can be operational at lower temperatures. Cleaning can also be carried out almost immediately since the equipment is cool. Moreover, during processing, heating conditions can be adjusted instantaneously and effectively.

As with all processing techniques, application of microwave energy for food processing has some limitations (Mudgett, 1986; Ohlsson, 1991; Saltiel *et al.*, 1995) which may or may not outweigh the potential advantages. There are several reasons why the development of this technology has not been convincing: mainly due to a lack of knowledge and understanding about the interaction of microwaves and food including information on product safety and quality, a serious consideration for any new technology, lack of communication between microwave engineers and food manufacturers, as well as a non-uniform heating characteristic. Buffler (1993) also provided several reasons for the failure of microwave food processing including: (1) economics since only 50 % efficiency can be expected compared with gas thus rendering microwaves advantageous only under some circumstances, (2) unfamiliar heating techniques and (3) poor perception due to operation safety, although it is not an issue. The main limitation has focused on the high investment cost, although the cost imbalance might not be as great if all the running costs are considered. Ohlsson (1991) pointed out that microwave heating is uneven and thus not suited for large foods although by careful engineering of the electromagnetic field and food product, it may be possible to minimize these effects.

To alleviate the problems realized from the limitations, a more in-depth understanding of the microwave heating principle and a good design of the microwave device that should be compatible to the application are essential. A flexible, proven and cost effective system is also required. Furthermore, the application of the microwave heating technique in conjunction with other heating techniques also provides better output in some cases including microwave finished drying. With the advent of these processes,

the problem can be greatly reduced. In addition, with the technological advances in microwave susceptors, problems such as lack of browning and crispness in microwave processed foods have been improved (Decareau, 1985).

Microwave applications in food processing

A number of microwave processes have been developed at both industrial and pilot scales (Decareau, 1985). Currently used microwave-based industrial food processing operations are summarized in Table 2.1.

Table 2.1 Major unit operations in microwave food processing.*

Application	Major objective	Estimated No.of industrial units	Products
Cooking	Modify flavor and texture	30	bacon, meat patties, poultry, sausage, sardines
Blanching	Inactivate spoilage enzymes	N.A.	vegetables, fruits
Drying	Reduce moisture content	30	pasta, onions, snack foods, fruit juices
Tempering	Raise temperature below freezing	200	meat, fish, poultry
Baking	Reduce undercooked core	N.A.	bread, donut proofing, proof-yeast-raised products
Pasteurization and Sterilization	Inactivate vegetative microbes, Inactivate microbial spores	100	fresh pasta, prepared meals, milk, sliced bread

*Adapted from Decareau and Peterson (1986), Mudgett (1989), Richardson (1991) and Giese (1992).
N.A.= Not Available

An extensive review of industrial applications has been covered in several publications (Copson, 1975; Mudgett, 1982; Decareau, 1985; Decareau and Peterson, 1986; Sanio and Michelussi, 1989; Ohlsson, 1991; Buffler, 1993). In this chapter, only relevant applications for pasteurization/sterilization are highlighted.

Pasteurization is a mild heat treatment given to bring up the temperature of foods. Primarily, this process kills vegetative pathogens of public health concern, inactivates enzymes and partly eliminates vegetative spoilage microbes present in the foods. Subsequently, chilled storage at above freezing point is necessary to minimize microbial growth (Lund, 1975). The main difference between pasteurization and sterilization is the level of temperature used to destroy microorganisms. Commercial sterilization involves more severe heat treatment designed at destruction of pathogens and reduction of spoilage to an economically feasible statistical level. It also relies on conditions created inside a container to render thermophilic microorganisms and spores to grow under conditions normally encountered during storage.

Microwave heat treatments have been ascertained to inactivate enzymes, eliminate microbial growth and retain quality attributes of the food products (Rosenberg and Bögl, 1987). Microbial destruction and enzyme inactivation by microwaves have been studied in a number of studies on various types of foods, including meat and meat products, poultry, egg and egg products, fish, shellfish, fruits and vegetables, sugar, beet molasses, pea protein concentrates, ready cooked meal, cereals, bread, cakes, pasta, starch, spices, soy milk as well as milk and milk products. A detailed review on pasteurization and sterilization has been covered by Mudgett and Schwartzberg (1982).

A number of successful processes have been reported for pasteurization of milk involving microbial destruction both in batch type (Chiu *et al.*, 1984; Merin and Rosenthal, 1984; Knutson *et al.*, 1988) and in continuous type (Hamid *et al.*, 1969; Jaynes, 1975). It was not until 1964, as reported in Decareau (1985), that the first successful experiment on microwave pasteurization was conducted on skim milk in glass jars at 915 MHz. Later, Hamid *et al.* (1969) carried out batch and continuous pasteurization of milk at 2450 MHz and reported that nearly complete destruction from total plate counts was obtained in a batch mode at 79.4°C for 12 s and 88.9°C for 14 s.

In their continuous mode, raw milk was allowed to flow by gravity through a 1.27 cm glass tube obliquely rigged across the waveguide at an 11° angle. They reported no survivors after heating to 82.2°C and that the destruction rate exponentially decreased.

Studies on milk pasteurization were also reported by Jaynes (1975) who conducted pasteurization in a continuous heat-hold-cool process at 2450 MHz. The pasteurization was achieved at 72°C for 15 sec. Sale (1976) performed pasteurization using a 5 kW, 2450 MHz microwave equipment to heat a free-falling stream of milk pressurized to 200°C for 40 ms and to prevent the build up of deposits. Although indistinguishable changes in flavor were reported, the process was too complex and costly.

Kudra *et al.* (1991) studied the heating behavior of several milk constituents under continuous-flow microwave heating. They reported that protein is a major contributor to microwave heating as compared with fat and lactose. Mudgett and Schwartzberg (1982) reported a successful system employing two frequencies to pasteurize yogurt and sour milk products in plastic cups with better heat uniformity (± 2°C) and extended shelf life.

Fruit juices and beverages have also been studied with respect to microwave heating as reported by Mudgett and Schwartzberg (1982). Several studies have been patented, e.g., fruit juices in PVC containers subjected to 2450 MHz microwaves at 60°C with external gas cooling to prevent softening; carbonated beverages continuously pasteurized at 2375 MHz microwaves under 4-6 atm pressure. Orange segments in heavy syrup packed in glass bottles have also been ascertained to be sterilized by microwaves at 83.5°C with better ascorbic acid retention (Lin and Li, 1971). There has been a clear advantage of microwave pasteurization of pulpy fruits and vegetable juices in glass or polypropylene bottles in terms of improved flavor and lower operating costs compared with conventional processes (Mudgett and Schwartzberg, 1982).

Pasteurization by microwaves has been industrially accomplished for decades, especially in Europe. Extended shelf life of the products from days to months appeared to be a major advantage over conventional pasteurization of pasta products. Fresh pasta is an example of a product in which conventional thermal pasteurization is ineffective due to its loose condition in nature in a package leading to relatively poor conduction heat transfer. The process is thus more effective using microwaves. There are a number of

microwave installations for pasta products in Europe. The process has also been successfully used for bread products, ready to eat meals, soft cheese, fruits and sugar mixture (Buffler, 1993). The equipment used for pasteurization consists of a long conveyorized tunnel microwave oven where the product is heated up by microwaves to the pasteurization temperatures.

A commercial microwave pasteurization for yogurt has been used in Germany employing two frequencies, 40 kW at 27 MHz and 8 kW at 2450 MHz. The low frequency technique increased the temperature of the main body of the container of yogurt while being conveyed through a water bath operating at 60°C. The high frequency technique heated the top portion of the products. This process provided extended shelf life (Decareau, 1985).

In Belgium and Germany, pre-packed pasta products have been commercially produced by a combined steam and microwaves for sterilization. A significant reduction in process time has been a major reason for improving the quality of the products (Ohlsson, 1991). Mudgett (1989) reported that microwave-sterilized milk and semi-solid foods in plastic pouches have been carried out at the U.S. Army Natick Research and Development Center and also at Alfa-Laval Co. in Sweden and are potentially commercialized. Decareau (1985) predicted that HTST microwave sterilization in individual package has an attractive future.

However, until recently, microwave heating has not been used for sterilization as successfully as for pasteurization (Buffler, 1993). The presence of hot and cold spots has been a major concern due to the ability of *C. botulinum* to grow readily in a nonacidic anaerobic condition (pH > 4.6). The process has not been commercialized in North America, however, microwave-sterilized pasta dishes have been successfully marketed in Europe.

Pasteurization of fruit juices

Spoilage of fruit juices is generally restricted to enzymes and microorganisms. Pasteurization of fruit juices is thus intended to serve two important functions: (1) inactivation of undesirable enzymes and (2) destruction of spoilage microorganisms.

Inactivation of enzymes: Pectin methylesterase (EC. 3.1.1.11)

Certain enzymes such as peroxidase, polyphenol oxidase and pectin methylesterase (PME) are generally present in fruit juices. These enzymes are capable of causing undesirable changes. Among these, PME is the dominant and the most heat resistant enzyme in several fruit juices. Typical pasteurization conditions for fruit juices established to inactivate PME and polygalacturonase are 65°C for 30 min, 77°C for 1 min and 88°C for 15 s (Ramaswamy *et al.*, 1992).

PME reaction with pectic substances causes cloud loss (Rouse and Atkins, 1952 and 1955) which is undesirable in certain juices especially citrus juices. The cloud loss is usually characterized by deesterification of high methoxyl pectin to low methoxyl pectin and precipitation of the latter as insoluble pectates in the presence of calcium ions. The process is also associated with the loss of characteristic citrus flavor due to the loss of lipids and dissolved essential oils (Veldhuis, 1977), the decrease in juice viscosity (Adams, 1991) as well as the increase in susceptibility to oxidation (Sajjaanantakul and Pitifer, 1991). The following diagram shows a pathway of cloud loss caused by PME:

$$\textit{Soluble Pectin} \xrightarrow{PME} \textit{Low Ester Pectin} \xrightarrow{Ca^{++}} \textit{Insoluble Pectates}$$

The published data have established that PME in citrus products contain multiple forms (Versteeg *et al.*, 1978; 1980; Wicker and Temelli, 1988). They differ by molecular weight or charge (Rillo *et al.*, 1992) and thus differ markedly in kinetic properties or thermal resistance (Versteeg *et al.*, 1980). Heat resistance also varies with variety, pH and soluble solid contents of the juice (Atkins and Rouse, 1953; Rouse and Atkins, 1953). It has been determined that thermal resistance of common bacteria and yeasts is generally less than that of PME (Eagerman and Rouse, 1976; Adams, 1991). The inactivation of PME, therefore, has been used as an index for pasteurization of citrus juices. After inactivation, PME is not able to regenerate (Nagy and Rouseff, 1986).

Destruction of microorganisms

Microorganisms are the major cause by which fruit juices are spoiled. The most common spoilage organisms in high-acid juices are fermentative yeasts, usually *Saccharomyces cerevisiae*, and lactic acid bacteria of the genera *Lactobacillus spp.* and *Leuconostoc spp.* (Swanson, 1989; Chen *et al.*, 1993) Yeasts produce undesirable CO_2 and ethanol, and lactic acid bacteria produce a buttery/buttermilk off-flavor due to diacetyl production (Chen *et al.*, 1993; Sharpe and Pettipher, 1983). Diacetyl level has been used as an indicator for spoilage during processing of citrus juices (Chen *et al.*, 1993). If the level of diacetyl exceeds 0.8 ppm, the processing line needs to be sanitized. It has been reported that *Lactobacillus* is the predominant spoilage bacteria (Rushing *et al.*, 1956; Swanson, 1989). Other species are implicated in the spoilage but to a lesser degree. Table 2.2 and 2.3 show genera of spoilage microorganisms isolated from citrus and apple products, respectively.

Table 2.2 Genera of spoilage microorganisms isolated from citrus products.*

Acetobacter	*Fonseceae*	*Proteus*
Alternaria	*Fusarium*	*Rhizopus*
Aspergillus	*Geotrichum*	*Rhodotorula*
Aurebasidium	*Gluconobacter*	*Saccharomyces*
Bacillus	*Hanseniaspora*	*Schwaniomyces*
Brettanomyces	*Hansenula*	*Serratia*
Byssochlamys	*Klebsiella*	*Sporobolomyces*
Candida	*Kloeckera*	*Torulaspora*
Citrobacter	*Lactobacillus*	*Torulosis*
Cladosporium	*Leuconostoc*	*Trichoderma*
Cryptococcus	*Mucor*	*Trichophyton*
Enterobacter	*Pichia*	*Xanthomonas*
Escherichia	*Penicillium*	*Xanthomonas*
		Zygosachharomyces

* Source: Chen *et al.* (1993).

Table 2.3 Genera of spoilage microorganisms isolated from apple products.*

Acetobacter	*Aspergillus*	*Byssochlamys*
Candida	*Cladosporium*	*Gluconobacter*
Kloeckera	*Lactobacillus*	*Leuconostoc*
Monilla	*Mucor*	*Paecilomyces*
Penicillium	*Rhodotorula*	*Saccharomyces*
	Torulopsis	*Zygosaccharomyces*

*Adapted from Swanson (1989).

The effectiveness of heat treatment depends on the initial load of microorganisms and the heat resistance of those microorganisms. Table 2.4 shows the heat resistance data for some spoilage microorganisms in fruit juices. The heat treatments substantially vary with product composition, volume, consistency and post-processing storage conditions. Garza *et al.* (1994) reported that *S. cerevisiae* was more sensitive when heated in peach puree (pH 3.9) than in buffer, but the pH (4 and 7) of buffer did not influence heat resistance. Yeasts are more sensitive to heat as the pH is decreased and the moisture content is increased (Deak and Beuchat, 1996). The heat resistance of microorganisms also depends on the growth stage whether they are vegetative cells or spores. Put and de Jong (1982) showed that ascospores of yeasts exhibited a considerably higher heat resistance than vegetative cells.

Theories on kinetics of thermal death of microorganisms have been detailed in literature (Farrell and Rose, 1967; Moats, 1971; Tomlins and Ordal, 1976). The microbial death rate is often logarithmic in nature exhibiting a first-order rate reaction kinetics which can be explained by a single-site theory (Charm, 1958) with death of microorganisms occurring from the inactivation of a single critical site per bacterial cell. Moats (1971), on the other hand, reviewed non-logarithmic survival curves and stated that the single-site theory cannot account for the initial lag or tailing. He suggested that the complex survival curves represented populations of different heat resistance and death occurred from inactivation of some fraction of multiple critical sites.

Table 2.4 Heat resistance of spoilage microorganisms.

Organism	Medium	D-value (min)	Temperature (°C)	Ref.
Yeasts				
Kluyveromyces spp.		0.1-0.2	60	1
S. cerevisiae		0.1-0.3	60	1
	apple juice (8°Brix)	0.9	55	2
	grapefruit serum	0.3-0.5	53	3
	orange juice	0.07	60	4
	apple juice	6.1*	60	4
Bacteria				
Lactobacillus spp.		1.0	60	5
L. plantarum	grapefruit serum	1.8-1.9	53	3
	tomato juice	11	70	6
L. casei	tomato juice	4	70	6
L. delbrueckii	tomato juice	5	70	6
L. Brevis	orange serum	1	60	7
Molds				
Byssochlamys spp.		120*	85	8
Aspergillus spp.		60*	85	5

* Ascospore

[1] Put and de Jong (1982), [2] Splittstoesser *et al.* (1986), [3] Parish (1991), [4] Shomer *et al.* (1994), [5] Splittstoesser *et al.* (1975), [6] Adapted from Tomlins and Ordal (1976), [7] Juven (1976), [8] Splittstoesser and Splittstoesser (1977).

Stumbo (1973) provided several causes for deviations from the logarithmic behavior of the survival curve that could arise from heat activation for spore germination, mixed flora, clumped cells, flocculation/deflocculation during heating nature of the subculture medium or anaerobiosis. Figure 2.3 shows four patterns of semi-logarithmic plot of survivors vs. time: (a) a convex survival curve showing initial lag period and followed by a logarithmic death rate, (b) a logarithmic survival curve, (c) a convex survival curve with tail and (d) a concave survival curve. The microbial death rate is often logarithmic in nature showing the first-order reaction kinetics. The survival curve after the initial lag is essentially logarithmic if the population of bacteria is homogeneous. The logarithmic survivors would result if each of the microbial cells were destroyed by inactivation of a single critical site (Charm, 1958) and the characteristic lag or shoulder would result if the cells are clumped (Stumbo, 1973). According to Gould (1995), the tailing behavior could result from either inactivation or resistance mechanisms as well as the heterogeneous population. The survival curve results in a concave fashion when the population is heterogeneous (Moats, 1971).

Inactivation/Destruction kinetics

Theoretical basis of thermal kinetics

Reaction kinetic parameters for thermal inactivation/destruction of enzymes and microorganisms as well as quality attributes are required for basically three reasons (Lenz and Lund, 1980): (1) for establishing a thermal process, (2) for minimizing loss of a quality factor and (3) for shelf life testing. The kinetic parameters available in the literature as described in Hayakawa *et al.* (1981) can be categorized into the following two groups: (1) those based on unistep or multi-step reaction kinetics employing application of some characteristic formulae and (2) those based on empirical analysis of time-temperature curves of the inactivation. The latter has been widely used for kinetic data analysis for thermal processing. The mechanism of inactivation/destruction of various attributes including enzymes and microorganisms can be based on the generalized nth-order kinetic model (Labuza, 1980):

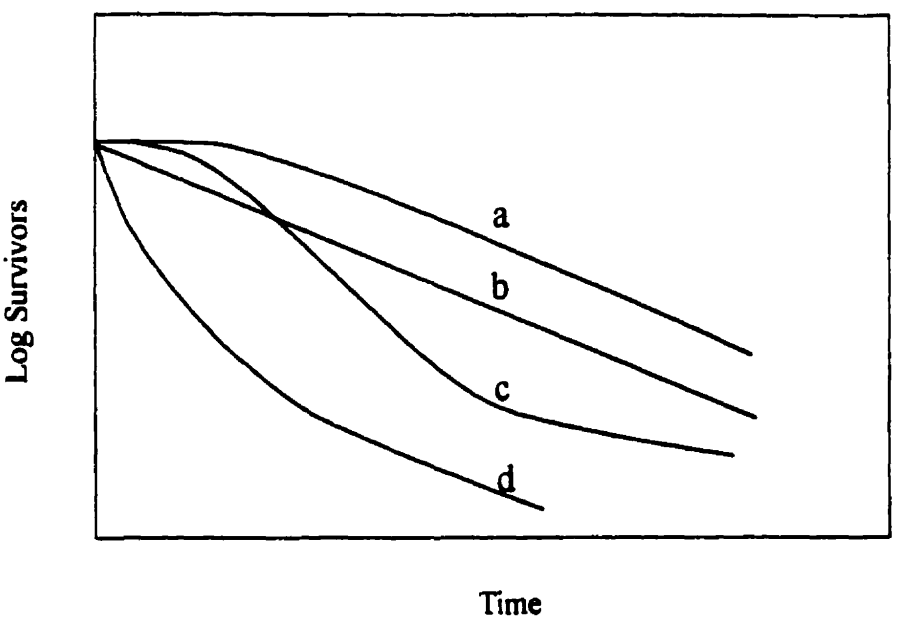

Figure 2.3 Types of survival curves of microorganisms (Source: Moats, 1971).

$$\frac{dC}{dt} = -kC^n \qquad (2.3)$$

where dC/dt = the time rate of change of concentration (C), k = the reaction rate constant, n = the order of reaction.

For many food components, enzymes and microorganisms, a first-order kinetics model adequately describes the destruction (Stumbo, 1973). However, according to Labuza (1982ab), nutrient losses due to thermal processing can be generally characterized to follow a zero- or first-order kinetics.

Considering a first-order reaction rate, integrating Eqn. (2.3) between limits C_1 at time t_1 and C_2 at time t_2 and converting the natural logarithm to base 10 results in:

$$\log C_2 = \log C_1 - \frac{k}{2.303}(t_2 - t_1) \qquad (2.4)$$

The temperature dependency of the reaction rate constant (k) can be described by many theoretical and experimental relationships (Adamson, 1986). Among those, two principal methods have been proposed. One of the relationships which was empirically introduced by Arrhenius in 1889 is based on a thermodynamic approach:

$$k = S \exp\left(\frac{-E_a}{RT}\right) \qquad (2.5)$$

where E_a = the activation energy for the reaction and R = the universal gas constant and S = the frequency factor.

Rearranging Eqn. (2.4) for two temperatures T_1 and T_2 with their corresponding reaction rate constants, k_1 and k_2, results in:

$$\log\frac{k_1}{k_2} = \frac{-E_a}{2.303R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right) \tag{2.6}$$

The Thermal Death Time (TDT) which is the alternate concept of describing the temperature dependency of the reaction rates in food that is based on empirical considerations proposed by Bigelow (Lund, 1975).

The D-value or decimal reduction time represents the time required to reduce concentration by 90%. This can be obtained from the semi-logarithm plot of logarithmic concentration vs. time as the time taken for the straight line portion to traverse one log cycle. The use of the D-value concept makes it easier to visualize the reduction of any particular food component at that time. The D-value is reciprocally related to k as follows:

$$D=\frac{2.303}{k} \tag{2.7}$$

The temperature dependency of k and D are, however, at variance when considering the TDT and Arrhenius concepts. The TDT approach is based on the assumption that the thermal death time (or D-values) of microorganisms, enzymes, or nutrients follows a semi-logarithmic relationship and relates proportionally with temperature as follows:

$$\log\frac{D_1}{D_2}=\frac{T_2-T_1}{z} \tag{2.8}$$

where D_1 and D_2 are D-values at T_1 and T_2, respectively. Since the D-value is based on a logarithmic destruction, the complete inactivation/destruction of enzyme activity or of the microbial population is theoretically not feasible. An adequacy of heat treatment therefore employed a probability approach for process calculation.

From Eqn (2.6) and (2.8), the relationship between E_a and z can be obtained as follows:

$$z = \frac{2.303RT_1T_2}{E_a} \qquad (2.9)$$

It should be noted that both TDT and Arrhenius concepts contradict each other since the reaction rate constant (k) is a reciprocal of the absolute temperature for the Arrhenius concept with E_a as the slope index of a semi-logarithmic plot. Whereas the D-value is a direct proportional to temperature in the TDT concept with z as the slope index of a semi-logarithmic plot. However, Lund (1975) suggested that the two concepts are reconcilable at small temperature ranges. According to Ramaswamy *et al.* (1989), both concepts have been proven to be suitable for studying inactivation/destruction kinetics; however, they demonstrated that erroneous results could be associated with conversion of parameters from one concept to another depending on associated reference temperature and temperature range employed. The authors recommended the use of the lower and upper limit of the experimental temperature range instead of the approach suggested by Lund (1975) where T is assumed to be proportional to 1/T for small temperature ranges. The TDT concept is more widely applied in thermal process calculation (Hallstrom *et al.*, 1988; Ramaswamy *et al.*, 1989)

Kinetic parameters can be influenced by several intrinsic factors including food composition, pH, water activity, soluble solids concentration as well as extrinsic factors which include temperature, time and method used for inactivation/destruction (Lund, 1975). In addition, assuming the linearlized z-values at considerably different temperatures from the experimental temperature has been cautioned and could lead to erroneous results (Burton, 1988; Hallstrom *et al.*, 1988; Datta, 1993).

Lag correction

To determine kinetic parameters of inactivation/destruction of many food components, the food components in appropriate media are subject to heat treatment at different temperatures. However, there is always a lag period associated prior to reaching the temperatures of the surrounding heating or cooling media. It is, therefore, essential to

properly correct the times associated during the lag periods especially when the heating time is fairly short. In some processes, a shorter lag period could be obtained by using steam as the heating medium which provides a better heat transfer. This, however, does not necessarily eliminate the lag corrections.

In a real process, the food component passes through changes in temperatures and the lethal effects of temperatures are integrated over the heating time based on the time-temperature profile giving a process lethality (F):

$$F = \int_0^t 10^{\frac{(T-Tref)}{z}} dt \qquad (2.10)$$

Most of the early literature relevant to PME inactivation discarded the heating lag correction by assuming instantaneous increase in temperatures (Rouse and Atkins, 1952; 1953; Atkins and Rouse, 1953; Ülgen and Özilgen, 1991). There are several reports showing application of the time corrections to the lag periods (Eagerman and Rouse, 1976; Nath and Ranganna, 1977abc; Versteeg *et al.* 1980; Ramaswamy and Ranganna, 1981; Marshall *et al.* 1985; Wicker and Temelli, 1988). Graphical and numerical integration methods have been employed in these studies. The accumulated lethality was integrated based on time-temperature history of the sample. The procedure is similar to the one described in Hayagawa *et al.* (1981) who suggested an iterative computerized procedure for estimation of kinetic parameters by correcting isothermal heating times for heating and cooling lags.

Microwave kinetics

As in thermal inactivation/destruction, microwave inactivation/destruction kinetics of food constituents such as enzymes, microorganisms and quality attributes are required for establishing microwave processing. Similar concepts can be applied for determining

kinetic parameters during microwave heating, however, non-isothermal heating conditions are involved in this case. The food constituent basically experiences changing temperature with time. The procedure is more complex than with the isothermal procedure. There have been only a few studies describing kinetics during microwave heating. A numerical integration of lethalities of *E. coli* inoculated in agar (Keenan, 1983) and apple juice (Foley, 1985) was used based on time-temperature profiles for predicting thermal inactivation effects during microwave heating.

Mechanisms of inactivation/destruction

The mechanisms contributing to microbial death or enzyme inactivation can be classified into 3 major groups: (1) thermal effects, (2) kinetic effects and (3) chemical effects.

Thermal effects

At the molecular level, the structural changes of the bound water layer may be affected by conventional thermal energy (Cleary and Mills, 1970). This also leads to changes in stability and functionality of macromolecules and subsequently the biological processes in the cells/molecules such as denaturation of proteins and nucleic acids inducing inactivation/destruction of enzymes or microorganisms.

Kinetic effects

The net charges of cells may be influenced in an electromagnetic field of microwaves and could lead to a rapid oscillation that exceeds the elastic limitation of the cell wall resulting in the disruption of the cell membrane (Carroll and Lopez, 1969; Khalil and Villota, 1988; Palaniappan *et al.*, 1990). This may lead to modification of membrane permeability, leakage of cellular contents such as release of bound water, loss of cell functionality and, eventually, death of cells. The passage of electron current could also induce the alteration of growth and nerve processes of cells (Cope, 1976). In addition, an alignment of micromolecules in the field which is sometimes referred to as the pearl chain formation may cause the breakdown of lipids and formation of free fatty acids (Teixeira-Pinto *et al.*, 1960; Wildervanck *et al.*, 1959; Olsen *et al.*, 1966; Rosen, 1972).

Chemical effects

The quantum energy levels of microwaves (Table 2.5) are several magnitudes lower than that required to break chemical bonds (Table 2.6), it is therefore unlikely that microwaves could break any type of chemical bond in foods (Rosen, 1972; Pomeranz and Meloan, 1987). However, it is likely that free radicals of oxygen, hydrogen, hydroxyl and hydroperoxyl may be formed by simultaneous absorption of energy (Olsen *et al.*, 1966; Rosen, 1972). Microbial cells may also be selectively heated by microwaves depending on their chemical composition and the surrounding medium (Carroll and Lopez, 1969). In view of food as a whole, it is possible that microwave energy may be concentrated in micro- or macro-scopic layers of food resulting in unconventional chemical reactions due to higher local temperature than the average temperature (Lorenz, 1976).

Table 2.5 Types of radiation with wavelength and quantum energy*

Radiation type	Typical wavelength (cm)	Quantum energy (eV)
Gamma rays	10^{-10}	1,240,000
X-rays	10^{-9}	124,000
Ultraviolet light	0.00003	4.1
Visible light	0.00005	2.5
Infrared light	0.01	0.012
Microwaves	10	0.000012
Radio	30,000	0.000000004

* Source: Rosen (1972).

Table 2.6 Quantum energy required for breakage of chemical bonds.*

Bond	Quantum Energy (eV)
H-OH	5.2
$H\text{-}CH_3$	4.5
$H\text{-}NHCH_3$	4.0
$H_3C\text{-}CH_3$	3.8
ph CH_2-COOH	2.4

* Source: Rosen (1972).

Non-thermal microwave effects

Since the middle of the 1920s, numerous studies have been carried out on the effects of microwave heating on food and its constituents and these have hastened the controversial issue on non-thermal microwave effects. Olsen *et al.* (1966) was probably the first who postulated the non-thermal effects of microwave heating. Several published studies have demonstrated that inactivation/destruction by microwaves cannot be solely explained by conventional thermal effects (Grecz et al., 1964; Olsen *et* al., 1966; Culkin and Fung, 1975; Chipley *et al.*, 1980; Dreyfuss and Chipley, 1980; Khalil and Villota, 1985). Some early work by Gray in 1968 (Decareau, 1985) led to the granting of patents for food sterilization using microwave energy at low temperatures. Most, among the relevant work between 1944 and 1983 as reviewed by Decareau (1985), have concluded that microbial inactivation/destruction is solely due to heating. Some early work attempted to determine the effects of microwaves without heating by exposing test samples to microwaves without the presence of water and reported no difference from conventional heating (Delany *et al.*, 1968; Vela and Wu, 1979). However, Vela and Wu (1979) suggested that water may play an important role in enhancing any additional microwave effects. Several studies have also been devoted to demonstrate the existence of non-

thermal microwave effects by controlling temperature below where thermal inactivation/destruction is predominant as reviewed in Chipley (1980). They reported no apparent lethal effects of microwaves at low temperatures. Some published data of studies investigating non-thermal microwave effects are presented in Table 2.7. The results have further stimulated this controversial issue since the information has been provided ambiguously and skeptically. Doubts have been mainly associated with the temperature measurement, temperature exposure of the samples, improper comparison of microwave and conventionally treated samples, and microwave exposure period.

Evidence with microorganisms

It is evident that low frequency alternating electromagnetic fields can induce biological changes in cells such as changes in the functioning of the nervous system that may ultimately disturb the function of the cell membrane. However, these frequencies are over 10 million times lower than microwave frequencies. Non-thermal effects of radio frequencies (RF) were demonstrated in the early work of Fleming (1944). Ponne *et al.* (1996) reported membrane damage of a simple cell model such as liposome vesicles at sublethal temperatures exposed to RF field at frequencies of 27 and 100 MHz, but no such effects were found in more complex cells like *Erwinia carotovora* cells. RF resulted in a change in biological membrane stability. Abramov *et al.* (1984) studied the effects of RF (250-500 MHz) on the metabolism of yeast cells and found that acetal production was induced by the RF treatment. However, contradictory results on the effects of RF have been reported in other studies (Brown and Morrison, 1954; Carroll and Lopez, 1969).

A number of other studies have investigated the existence of non-thermal effects. Goldblith and Wang (1967) concluded from experiments on heating of aliquotes of *E. Coli* and *B. subtilis* by microwave and conventional methods, that only thermal effects contributed to the destruction of the spores since there were no markedly differences in the survival of spores for the same time-temperature exposure. In contrast, Olsen *et al.* (1966) showed that *Fusarium* spores can be inactivated at low temperatures using microwaves as compared with conventional heating. Lechowich *et al.* (1969) introduced

Table 2.7 Published data of studies investigating microwave effects on microorganisms, enzymes, and nutrients.

Test sample	Frequency (MHz)	Additional Effects	Reference
Microorganisms			
C. sporogenes	2450	Yes	Grecz *et al* (1964)
Fusarium solani, Fusarium phaseoli			
	2450	Yes	Olsen *et al.* (1966)
E. coli, B. subtilis	2450	No	Goldblith and Wang (1967)
S. faecalis, S. cerevisiae			
	2450	No	Lechowich *et al.* (1969)
S. cerevisiae, E. coli, B. subtilis			
	60	No	Carroll and Lopez (1969)
E. Coli, S. typhimurium			
	915	Yes	Culkin and Fung (1975)
Active dry yeast, *E. Coli*			
	2450	No	Vela and Wu (1979)
S. aureus	2450	Yes	Dreyfuss and Chipley (1980)
B.stearothermophilus	2450	Yes	Khalil and Villota (1988)
E. coli	2450	No	Fujikawa *et al.* (1992)
Cl. sporogenes	2450	No	Welt *et al.* (1994)
Enzymes			
Peroxidase, Polyphenol oxidase, Pectinesterase, Catalase, Alpha amylase			
	60	No	Lopez and Baganis (1971)
Glucose-6-phosphate dehydrogenase			
	2.8 GHz	No	Belkhode *et al.* (1974a)

Table 2.7 ... Continued

Test sample	Frequency (MHz)	Additional Effects	Reference
Peroxidase	2450	Yes	Henderson *et al.* (1975)
Peroxidase, PPO	2450	Yes	Lorenz (1976)
Lipoxygenase, Trypsin inhibitor	2450	Yes	Esaka *et al.* (1987)
Lipoxygenase	2450	Yes	Kermasha *et al.* (1993a)
Lipase	2450	Yes	Kermasha *et al.* (1993b)
Nutrients			
Thiamine	2450	No	Goldblith *et al.* (1968)
Thiamine	2450	No	van Zante and Johnson (1970)
Thiamine	2450	No	Welt and Tong (1993)

S. faecalis and *S. cerevisiae* cells to microwave exposure in a similar fashion and showed no lethal effects of microwaves other than those contributed heat. However, using a similar instrumentation as Lechowich, Khalil and Villota (1988) investigated the viability of *S. aureus* cells but found that there was less cell viability for microwave-treated cells, thus supporting the presence of unknown non-thermal effects.

A review by Lorenz (1976) concluded that there was sufficient evidence for stating that microwave interactions can be explained solely by thermal effects. Fujikawa *et al.* (1992) showed that the microwave destruction profile of *E.coli* was similar to that of conventional heating by considering the same temperature increase during heating for comparison. In addition, Welt *et al.* (1994) reported no additional effects other than heat contributed from microwave heating of *C. sporogenes* spores. However, the spores were not exposed to microwave heating continuously since temperatures were controlled by an on and off mode whereby during the off period temperatures were maintained by thermal.

On the other hand, Culkin and Fung (1975) found additional effects of microwaves at 915 MHz on the destruction of *E. coli* and *S. typhimurium* in microwave cooked-soup (batch heating) and reasoned that some of the apparent non-thermal effects may be exerted through molecular level responses of the biological materials to the generated thermal energy. It was suggested that the thermal effects were contributed from generated heat and non-thermal effects were apparent since there were greater decreases in survival in the top region than those in the middle and bottom regions. Such decreases may arise from the higher microwave intensity in the top region than in the other regions. In addition, Dreyfuss and Chipley (1980) demonstrated the non-thermal effects of microwave heating on specific activities of some selected enzymes from *S. aureus*.

Evidence with enzymes

Enzyme inactivation by microwave heating has been reported as a consequence of increases in temperature. Very limited information relating to microwave inactivation of enzymes in food systems is available in the literature. However, Lopez and Baganis (1971) studied inactivation of several enzymes including peroxidase, polyphenol oxidase, pectin methylesterase, catalase and alpha amylase and found no apparent effects of RF

at 60 MHz. Henderson *et al.* (1975) studied inactivation of horseradish peroxidase using 2450 MHz microwaves by circulating carbon tetrachloride as a coolant to control the temperature of the sample (0.8 mL) at ~ 25°C. They reported significant enzyme inactivation at high absorbed power (> 125 W/cm^3 for 20 min or > 60 W/cm^3 for > 20 min) and reasoned that protein denaturation may be due to local heat generation within the sample. As reviewed in Lorenz (1976), the microwave inactivation pattern of peroxidase and polyphenol oxidase in potato was similar to that obtained for conventional blanching, but at a faster rate. Esaka *et al.* (1987) investigated the inactivation of lipoxygenase and trypsin inhibitor in winged bean seeds by microwave heating and compared the results with those from conventional heating. They concluded that microwave heating was more effective, however, the effectiveness of come-up time was not considered in their studies. Kermasha *et al.* (1993ab) studied the effect of microwaves on wheat germ lipase and soybean lipoxygenase and suggested that enhanced effects associated with microwave heating may be due to the interaction of polar parts of enzymes with the alternating electromagnetic field of microwaves. In their experiment, equivalent time-temperature treatments were accommodated for comparison between microwave and conventionally treated samples.

Evidence with nutrients

Critical reviews on effects of microwaves on nutritive values of foods have been given by Lorenz (1976) and Cross and Fung (1982). Some studies have been carried out on thiamine degradation by microwaves at sublethal or low temperatures. Goldblith *et al.* (1968) exposed a certain amount of thiamine solution to microwaves while maintaining the sample temperature constantly low with the circulation of cool kerosene coolant through the jacket of the condenser. They reported no destruction of thiamine from microwave heating at 33°C for 15 and 30 min demonstrating no apparent non-thermal effects. Welt and Tong (1993) reported no significant difference between thiamine degradation kinetics obtained from microwave and conventional heating. However, in their experiment, microwave power was only 50% of the fully operated power meaning that during the heating process, it was an effect of combined microwave and thermal.

Summary of the literature review

It is now well established that microwaves can be effectively used for food processing. However, to introduce microwaves for pasteurization of fruit juices, interaction of microwaves to the juice and its components including enzymes and microorganisms need to be well understood. Moreover, serious attention has not been devoted to establishing kinetic data of enzyme inactivation and microbial destruction for microwave pasteurization purposes and the controversial issue on non-thermal microwave effects has not been adequately examined. It may be suggested that systematic experiments for proper comparison should be carried out to determine and compare the kinetic data for microwave pasteurization and reveal the existence of non-thermal microwave effects.

CHAPTER III

CONVENTIONAL THERMAL KINETICS OF ENZYME INACTIVATION AND MICROBIAL DESTRUCTION

ABSTRACT

Kinetics of thermal inactivation of enzyme pectin methylesterase (PME) in single-strength orange juice and destruction kinetics of two selected spoilage microorganisms, *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, in single-strength apple juice were evaluated in the pasteurization temperature range of 50-90°C. Aliquots of test samples containing (a) enzyme enriched orange juice and (b) inoculated apple juice were subjected to various heat treatments in a well-stirred water bath. Based on residual PME activity as well as survival numbers of microorganisms, first-order rate, kinetic parameters were evaluated. Effectiveness of come-up and come-down periods (lag) were taken into consideration based on time-temperature history of test samples. PME inactivation studies in orange juice (pH 3.7) indicated the presence of two fractions: a heat sensitive fraction with D-values of 155, 37, 8.7, 6.7 and 2.9 s, and a heat resistant fraction with D-values of 1240, 1200, 893, 329 and 109 s at 60, 70, 80, 85 and 90°C, respectively. The corresponding z-values were 17.7 and 31.1°C, respectively. PME was more sensitive to thermal inactivation at higher soluble solids concentration and lower pH. Microbial destruction in apple juice (pH 3.4) gave D-values of 58, 25, 10 and 1.9 s at 50, 55, 60 and 70°C, respectively, for *S. cerevisiae* (ATCC 16664) and 52, 22, 8.4 and 1.2 s at 55, 60, 70 and 80°C, respectively, for *L. plantarum* (ATCC 14917). The corresponding z-values were 13.4 and 15.9°C, respectively.

INTRODUCTION

Pasteurization, a mild heat treatment, is given to fruit juices and beverages to control the activity of undesirable enzymes as well as spoilage microorganisms. The

heating conditions may vary depending upon the products. For citrus juices, the traditional pasteurization procedure is to subject the juice to 91°C for 7 s (Nagy and Rouseff, 1986) and, for apple juice, it ranges between 77°C and 88°C with times of 25 and 30 s (Moyer and Aitken, 1971). These treatments have been recognized to cause some heat damage to the delicate flavor of these products, and therefore, alternate processes which retain the fresh-like quality of fruit juices are continually being explored.

Kinetic data on enzyme inactivation or microbial destruction are used for establishing the minimal pasteurization schedule. The usefulness of kinetic data depends on the accuracy of gathered data and proper accounting of thermal contributions of come-up (CUT) and come-down (CDT) times (lag). The majority of published data on enzyme or microbial thermal resistance may have limited scope because of lack of accounting of lethal contributions of come-up and come-down times.

Pectin methyl esterase (PME) is the predominant and undesirable enzyme in citrus juices such as orange juice. PME (E.C.3.1.1.11) is also referred as pectinesterase, pectase and pectin-methoxylase (Rouse and Atkins, 1955). PME hydrolyzes ester bonds of pectin in citrus juices and, as a result, the juice looses its stability. The enzyme has been implicated in the cloud loss of citrus beverages (Rouse and Atkins, 1952; 1955). Thermal resistance of PME has been recognized to be greater than that of common bacteria and yeasts in citrus juices (Eagerman and Rouse, 1976; Adams, 1991) and, hence, PME inactivation has been used to determine the adequacy of pasteurization.

The effects of pH and soluble solids concentration on the thermal inactivation kinetics of PME have been studied in different varieties of oranges (Rouse and Atkins, 1953; 1955; Atkins and Rouse, 1953; 1954). Rouse and Atkins (1952) reported shorter holding times for PME inactivation in orange and grapefruit juices containing 5% pulp as compared with those with 10% pulp. Atkins and Rouse (1954) also showed higher inactivation rates at lower solids concentration as well as at lower pH of orange juice. Nath and Ranganna (1977b, c; 1983) developed thermal processes based on inactivation of PME and reported z-values for PME inactivation in various products: 11.7°C and 16.0°C in acidified canned musk melon and guava syrup; 10-12°C in mandarin orange depending on pH and soluble solids content. Eagerman and Rouse (1976) established

pasteurization conditions for citrus juices as F_{90} = 1 min (z = 12.2°C) and F_{90}= 1 min (z = 5.2°C) for grapefruit juice.

Versteeg *et al.* (1980) showed three forms of PME in Navel oranges, one of which (the high molecular weight PME) was reported to be the most heat resistant fraction with a z-value of 6.5°C and a D-value of 24 s at 90°C and of the other two forms of PME isozymes; one was more rapidly inactivated (D_{60} of 47 s) than the other (D_{70} of 27 s) with z-values of 11°C and 6.5°C, respectively. Deviations from first order kinetics of thermal inactivation were reported to be due to different thermostabilities. Wicker and Temelli (1988) also reported two fractions of PME in orange juice with D-value (90°C) and z-value for heat sensitive fraction as 0.225 s and 10.8°C, respectively, and for heat resistant fraction as 32 s and 6.5°C, respectively. These studies indicate that PME inactivation kinetics depend on several factors: variety and composition (acidity, soluble solids, pulp content) of juice as well as heating method.

For apple juice, use of PME inactivation as a criterion is not a common practice. Inactivation of alternative enzymes such as polyphenol oxidase (PPO) which causes enzymatic browning and is known to be abundant in apple juice could be promising. However, PPO is not very heat resistant (Vámos-Vigyázó, 1981; Adams, 1991), and therefore, not used as an index for pasteurization. Destruction of microorganisms was thus chosen as an index for establishing microwave pasteurization schedule of apple juice.

Growth of microorganisms in apple juice is well documented and is typified by production of off-flavors and product spoilage. The microbiological spoilage of fruit juices, especially apple juice, have been implicated to yeasts, molds and lactic & acetic acid bacteria (Swanson, 1989). Spore-forming bacteria have not been a major concern in the spoilage of apple juice products since they normally propagate only at pH > 4.5 (von Schelhorn, 1951). The lactic acid bacteria (*Lactobacillus* spp.) impart an undesirable buttermilk-type flavor to the juice due to the production of diacetyl as a volatile metabolic end product. Spoilage of apple juice from yeasts is primarily confined to fermentation by *Saccharomyces* spp. although other yeasts are also capable of causing spoilage. *Saccharomyces* spp., especially *S. cerevisiae* causes fermented off-flavors due to the production of carbon dioxide, ethanol and trace of other fermentation products.

The objectives of this study were primarily to determine inactivation/destruction kinetics (D and z-values) of PME in orange juice and *S. cerevisiae* and *L. plantarum* in apple juice during thermal heat treatments in the pasteurization temperature range (50 to 90°C), especially with consideration of come-up and come-down time effectiveness. The primary purpose was to have a data base on thermal inactivation for later comparison with those obtained under microwave heating conditions.

MATERIALS AND METHODS

A. PME IN ORANGE JUICE

Enzyme/substrate preparation

Commercial citrus PME enzyme (Sigma Chemical Co., St. Louis, MO) was used to prepare the enzyme stock in 1.70 mol/L NaCl solution (125 PME unit/mL). Citrus pectin (Sigma Chemical Co., St. Louis, MO) was used as a substrate. Ten grams of citrus pectin was mixed with 11.7 g NaCl and then slowly added, with constant stirring, to 800 mL of distilled water and made up to 1 L. Toluene (4-6 drops) was added as a preservative (Rouse and Atkins, 1955) and the substrate was kept at 4°C until use. Prior to enzyme assay, the substrate was warmed up to 30°C and adjusted to pH 7.5 with 0.2 mol/L NaOH.

Orange juice preparation

Fresh squeezed orange juice (Old South Brand) (pH 3.7, 11.7°Brix) was bought in bulk and kept frozen (-10°C to -15°C) until use. A calculated amount of PME stock solution (18 mL) was added to 400 mL of heated juice (with no residual PME activity) in order to adjust the initial enzyme concentration to about 4-6 PME unit/mL. The pH was adjusted to 3.2, 4.2 and 4.6 using citric acid or NaOH as necessary. For higher soluble solids content (20 and 41°Brix), frozen concentrated orange juice (from local market) was used and enriched by commercial PME, as found necessary.

Pectin methyl esterase assay

PME activity was determined by titrating the liberated carboxyl group at pH 7.5 (30°C) using the method adopted by Rouse and Atkins (1955). The activity was expressed as PME unit/mL which represented the μ-equivalents of acid liberated per min per mL at pH 7.5 and 30°C. The slightly modified procedure consisted of adding 2.0 mL of juice sample to 50 mL of pectin substrate (previously adjusted to pH 7.5) with constant stirring and quickly adjusting pH to 7.5 with 0.2 mol/L NaOH. The reaction was initiated as soon as the pH was adjusted to 7.5. The amount of NaOH (0.02 mol/L) used was recorded during the reaction period of 30 min. During the entire titration, a constant temperature of 30°C was maintained. PME activity was then calculated as follows:

$$\text{PME unit} = \frac{(\text{mL of 0.02 mol/L NaOH})(\text{mol/L of NaOH})(1000)}{(\text{mL of sample})(\text{time in min})} \quad (3.1)$$

Conventional thermal treatment

Aliquots of PME enriched orange juice (2 mL) were sealed in glass ampoules (13 mm O.D., 100 mm length) and subjected to heat treatments in a well-agitated water bath (Fisher Scientific, Ltd., Montreal, PQ) at selected temperatures for various times. Following heating, they were immediately cooled in an ice-water bath. Residual enzyme activities were then determined as detailed earlier. Temperatures employed for heat treatments were 60, 70, 80, 85 and 90°C at pH 3.7. The effects of pH (3.2, 4.2 and 4.6) and soluble solids content (20 and 41°Brix) were studied at 85°C. All treatments were duplicated.

Time-temperature profile determination

Time-temperature data during come-up (CUT) and come-down (CDT) times of test samples in the tubes were gathered during conventional thermal treatment using a 0.381 mm diameter copper-constantan thermocouple (Omega Engineering Inc., Stamford, CT) inserted into the juice in the tube. Temperatures were computer recorded via a data-logger (Dash-8, Metra-Byte Corp., Taunton, MA).

Kinetic data handling

Isothermal heating conditions:

Inactivation of PME has been shown in several studies to follow first order reaction kinetics indicating a logarithmic order of destruction. Mathematically, it can be expressed as:

$$\log_e\left(\frac{A}{A_i}\right) = -kt \qquad (3.2)$$

where: A = mean residual enzyme activity (PME unit/mL); A_i = mean initial enzyme activity (PME unit/mL); k = reaction rate constant (s^{-1}) at a particular temperature; t = inactivation time (s). The k-value was obtained as the negative reciprocal slope by regression of $\log_e(A/A_o)$ vs t. The decimal reduction time (D-value), defined as the heating time required to result in 90% inactivation of initial activity at a given temperature, can be obtained from k:

$$D=\frac{2.303}{k} \qquad (3.3)$$

The temperature sensitivity of D-values, expressed as z-value, was then determined as the negative reciprocal slope of log D vs. T regression. The z-value represents a temperature difference required to result in a ten-fold change in D-values. Mathematically:

$$z=\frac{(T_2-T_1)}{\log D_1 - \log D_2} \qquad (3.4)$$

where T_2 and T_1 are temperatures corresponding to D_2 and D_1.

Non-isothermal heating conditions: CUT and CDT corrections

In most thermal processing situations, food products packaged in cans are subjected to non-isothermal heating conditions. The lethality (F) accumulated at the cold spot (or hot spot during the CDT) is generally obtained by integration of the lethal effects of the temperature profile during the come-up, hold and come-down periods using the relationship:

$$t_e = F = \int_0^t 10^{(T-T_{ref})/z} dt \tag{3.5}$$

where T_{ref} is the reference or bath temperature. The accumulated lethality (F) is expressed as the effective time, t_e, at a reference temperature.

In thermal inactivation studies involving non-isothermal heating conditions, the contribution of the CUT and CDT is likewise added to the heating time by calculating the effective portion of the CUT and CDT using a similar concept. Computation of the effective time or lethality requires data on z-value which needs to be obtained from a regression of log D-value vs. temperature. For this purpose, first estimates of the D-values associated at the various temperatures (T) are obtained using uncorrected heating times assuming isothermal heating conditions. These values are used to calculate the z-value by regression of log D vs. T as the negative reciprocal slope. This z is then used to calculate the effective time (t_e) or accumulated lethality (F) under each condition using Eqn. (3.5) from which corrected D-values are calculated. These D-values are then used to get a new z-value which is then used again to get more precise values of effective times and used to recalculate the D-values and subsequently another z. The process generally is repeated several times for the convergence of D and z-values. A schematic flow chart of calculation steps is shown in Figure 3.1 (Nath and Ranganna, 1977abc; 1983; Ramaswamy and Ranganna, 1981; Awuah *et al.*, 1993). The kinetic values of heat resistant fraction were computed once all the times were corrected.

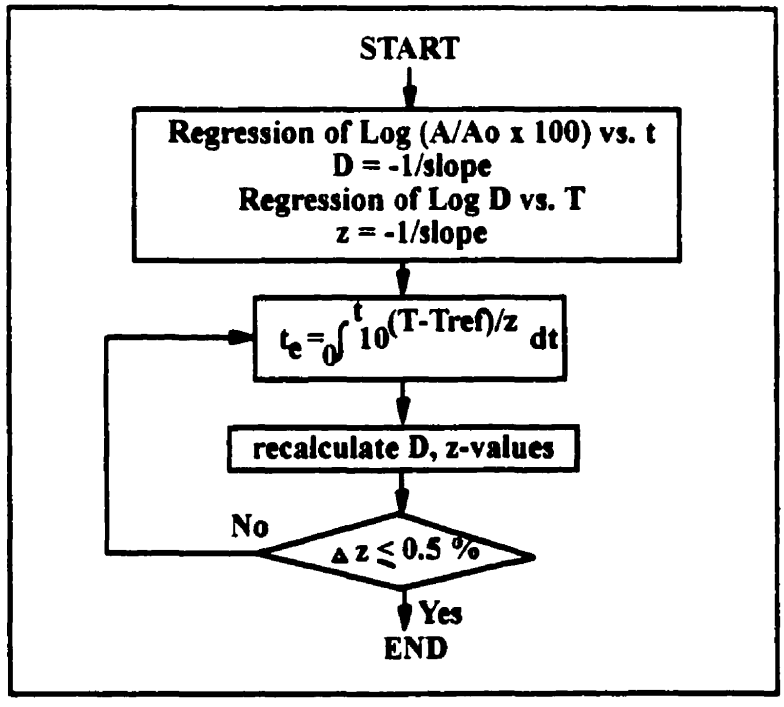

Figure 3.1 Flow chart of calculation steps for kinetic data analyses.

The accuracy of the accumulated lethality (F or t_e) calculated for a thermal process using numerical integration technique is generally dependent on the number of data points included in the calculation (Ross *et al.*, 1979). It should be, therefore, noted that since t_e or F is the area under time-lethal rate profile, it is necessary to include as many data points as possible for better estimation. A Fortran program was written (Appendix A) for calculating effectiveness fraction of the CUT and/or CDT by generating more data points (1000 points), based on linear interpolation in temperature, between successive time intervals of the gathered time-temperature profile .

B. MICROBIAL DESTRUCTION IN APPLE JUICE

Microbiological procedures

Freeze-dried cultures of *Saccharomyces cerevisiae* (ATCC 16664) and *Lactobacillus plantarum* (ATCC 14917) commonly present in apple juice were obtained from American Type Culture Collection (ATCC, Rockville, MD). They were rehydrated in 0.1% sterile bacto peptone water (Difco Laboratories, Inc., Detroit, MI). *S. cerevisiae* was plated on a potato dextrose agar (PDA) (Becton Dickinson and Company, Cockeysville, MD) acidified to pH 3.5 with 10% tartaric acid and incubated aerobically at 25°C for 72 h. Similarly, *L. plantarum* was plated on MRS agar (Difco Laboratories, Inc., Detroit, MI) and incubated anaerobically at 29°C for 72 h. A pre-culture was prepared by transferring a colony of each culture into 10 mL of malt extract broth and MRS broth (Difco Laboratories, Inc., Detroit, MI) which were selected as the growth media, respectively and incubated for 18 to 24 h at 25°C for *S. cerevisiae* and 29°C anaerobically for *L. plantarum*. The pre-cultures were then subcultured in 100 mL of the respective selected growth medium and incubated for another 18 to 24 h to reach the logarithmic growth phase. The cultures were harvested using a Beckman J2-21centrifuge (Beckman Instruments, Inc., Palo Alto, CA) with a JA-10 rotor for 25 min at 4°C and 4420 x g. Pellets were resuspended in 10 mL of 0.1% sterile peptone water and stored refrigerated at 4°C until further use within 24 h. The microbial population of these pre-cultures were enumerated by counting colony-forming units (CFU) using a surface spread-

plate technique prior to inoculation of the juice. The enumeration was performed by serial dilution of the cultures with 0.1% sterile bacto peptone water and by plating 0.1 mL of the test sample on an appropriate agar. The average viable counts in the pre-cultures of *S. cerevisiae* and *L. plantarum* were in the order of 10^8 CFU/mL and 10^{10} CFU/mL, respectively. Colonies were counted from plates of three appropriate dilutions such that the counts were in the range of 30-300 CFU/mL on a given plate, and using the dilution factors, the microbial concentrations were computed.

Apple juice preparation

Commercial pasteurized apple juice with pH of 3.4 obtained from A. Lassonde, Inc. (Rougemont, Québec) was inoculated with the enriched precultures of *S. cerevisiae* and *L. plantarum* inoculum to obtain initial viable counts of 10^5 to 10^6 CFU/mL.

Conventional thermal treatment

Two mL aliquots of cultured apple juice in 13 x 100 mm (diameter x length) test tubes were subjected to heat treatments in a well-stirred water bath (Neslab RTE 221, Neslab Instrument, Inc., Newlington, NH) operating at selected temperatures (50-70°C for yeast and 55-80°C for bacteria) for 10 s to 70 min, cooled immediately in an ice-water bath, and enumerated. All treatments were duplicated. The CUT and CDT time-temperature profiles of test samples in the tubes were determined and accommodated in the data analyses as detailed earlier.

RESULTS AND DISCUSSION

A. PME IN ORANGE JUICE

PME activity in fresh orange juice

The PME activity in the commercially fresh orange juice obtained from local market varied between 0.5 to 2 unit/mL. For the thermal kinetics studies, the original PME in the orange juice was first inactivated by heating the orange juice to boiling and

quickly cooling. Commercial PME was then added to the heat treated juice as typically employed in other studies (Eagerman and Rouse, 1976; Versteeg *et al.*, 1980; Marshall *et al.*, 1985; Wicker and Temelli, 1988) giving an initial PME activity of about 4 unit/mL

Come-up and come-down profiles and correction

Come-up time (CUT) effectiveness has been traditionally taken to be 42% of CUT as originally suggested by Ball (1923) for thermal process calculations. When the heating times are higher than CUT (isothermal heating conditions), a single correction factor accommodating the effective portion of CUT will suffice, and the total heating times are reduced by the ineffective portion of CUT. However, when the heating times involved are less than CUT (non-isothermal heating conditions), the full-CUT effectiveness may lead to overestimation of effective time. For example, a short time heating of 10 s at 90°C will yield an effective heating time of 4.2 s with a 42% CUT effectiveness approach; but in reality its effectiveness may be far less (Table 3.1). For such short-time (less than CUT) heating, the effectiveness can only be obtained by estimating the effective thermal time using Eqn. (3.5). Figure 3.2 shows a typical come-up period time-temperature profile under conventional thermal heating condition, the resulting lethal rate or thermal time curve and the effectiveness of CUT calculated as the ratio of the area under the lethal rate curve and a hypothetical rectangle indicating a step-change heating to the operating condition. The CUT temperature profile during the thermal heating was somewhat logarithmic in nature. The effectiveness during heating increased with time up to CUT. The experimental effectiveness of full length come-up periods varied from 46 to 58%.

A coupled come-down temperature profile is also illustrated in Figure 3.2 for the same condition. The temperatures decreased rapidly and reached levels where thermal inactivation was negligible within about 3-5 s in all the cases. Considering only the corrections due to the CDT, the effective portion was calculated as 0.4 s relative to effective times ranging from 40 to 300 s under the various heating conditions. The correction due to CDT was therefore much smaller as compared with that during the CUT, however it was included for computation of kinetic data as defined in Table 3.1.

Table 3.1. Heating conditions and CUT & CDT corrections for thermal inactivation of PME.

Temp. (°C)	Average CUT (s)	Uncorrected Heating Time (s)	Effectiveness of CUT (%)	CUT Correction (s)	CDT* Correction (s)
60	84	60	46	-32	+0.34
		84	58	-35	+0.38
		90 - 360	58	-35	+0.38
70	78	20	8.0	-18	+0.05
		45	26	-33	+0.18
		60	38	-37	+0.32
		78	50	-39	+0.38
		80 - 180	50	-39	+0.38
80	84	10	0.28	-9.9	0
		22	2.7	-21	+0.02
		33	9.9	-30	+0.10
		45	19	-36	+0.18
		60	31	-41	+0.27
		84	46	-45	+0.38
		92 - 180	46	-45	+0.38
85	100	10	0.23	-9.9	0
		20	1.3	-19	+0.01
		30	4.9	-28	+0.05
		45	14	-39	+0.15
		60	24	-45	+0.21
		90	42	-52	+0.34
		100	47	-53	+0.40
		120 - 180	47	-53	+0.40
90	98	15	2.2	-14	+0.02
		30	9.1	-27	+0.08
		45	20	-36	+0.20
		60	31	-41	+0.28
		75	41	-44	+0.36
		98	53	-45	+0.43
		105 - 180	53	-45	+0.43

* CDT ranged from 3-5 s.

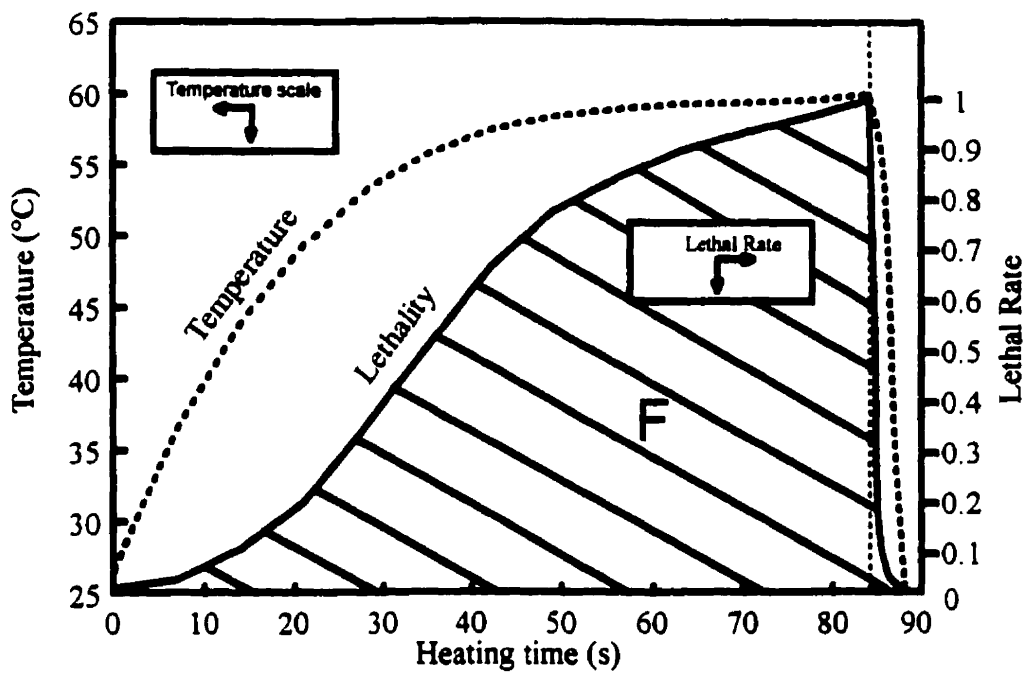

Figure 3.2 A typical time-temperature profile and corresponding lethal rate profile during thermal inactivation.

Thermal inactivation kinetics

Figures 3.3a and 3.3b show thermal inactivation curves at various temperatures (pH 3.7, 11.7°Brix) with corrected (effective) times on x-axis. Although upon first look, the plot (Figure 3.3a) appears to be curvilinear, it could easily be resolved into two first-order rate curves, the first one much steeper than the second one and disappearing within a short heating time. The net result is identification of two fractions of PME, i.e., one more heat sensitive than the other. Such a behavior has been observed in other studies (Eagerman and Rouse, 1976; Versteeg *et al.*, 1980; Wicker and Temelli, 1988). The time scale from Figure 3.3a was enlarged showing the exploded view of the inactivation kinetics of heat sensitive fraction in Figure 3.3b. The calculated kinetic parameters (D-values) of PME in orange juice (Table 3.2) obtained from the negative reciprocal slopes of linear sections of the different curves for both uncorrected and corrected.

Table 3.2. Thermal kinetic parameters (D and z-values) of PME in orange juice at various temperatures (pH 3.7 and 11.7°Brix).

Temperature (°C)	D-value (s)			
	Heat Sensitive Fraction			Heat Resistant Fraction
	Uncorrected	CUT Corrected	CUT + CDT Corrected	Corrected[2]
60	199 (0.99)[1]	154 (0.99)	155 (0.99)	1240 (0.94)
70	81.3 (0.89)	37.2 (0.92)	37.5 (0.92)	1200 (0.92)
80	49.3 (0.95)	8.45 (0.93)	8.67 (0.93)	892.8 (0.99)
85	37.4 (0.88)	6.53 (0.87)	6.69 (0.88)	328.9 (0.96)
90	27.7 (0.98)	2.85 (0.92)	2.92 (0.92)	109.3 (0.99)
z value (°C)	**36.2 (0.98)**	**17.6 (0.99)**	**17.7 (0.99)**	**31.1 (0.87)**

[1] The values in parentheses are the coefficients of determination (R^2).
[2] The CDTcorrections did not affect D-values.

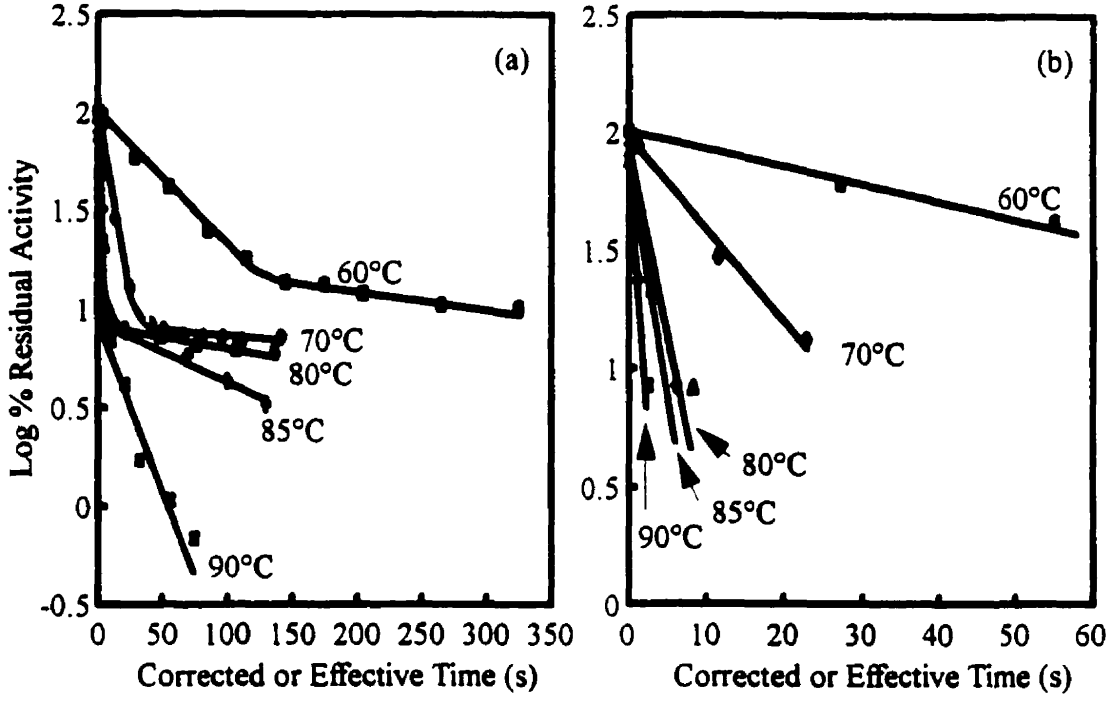

Figure 3.3 Thermal inactivation curves of PME in orange juice showing (a) both heat sensitive and heat resistant fractions and (b) an exploded view of the inactivation of heat sensitive fraction.

Temperature sensitivity plots of inactivation rates for both heat sensitive and heat resistant fractions of PME are shown in Figure 3.4. The z-values were 17.7°C and 31.1°C for the heat sensitive and heat resistant (less heat sensitive) fractions, respectively. It was found that only a small percentage of activity [8% in this study and 5% as reported by Versteeg *et al.* (1980)] is contributed by the more heat resistant fraction.

Influence of soluble solids concentration and pH

Thermal inactivation of PME at 85°C as influenced by soluble solids concentrations is shown in Figure 3.5 for both heat sensitive and heat resistant fractions with an exploded view of the plot shown for the heat sensitive fraction in Figure 3.5b. The calculated D-values are summarized in Table 3.3.

Table 3.3. Corrected decimal reduction times of PME in orange juice at various soluble solid concentration, 85°C and pH 3.7.

Soluble Solids (°Brix)	D-value (s)			
	Heat Sensitive		Heat Resistant	
11.7	6.69	(0.88)*	329	(0.96)
20	5.34	(0.94)	241	(0.94)
41	2.60	(0.96)	201	(0.88)

* The values in parentheses are the coefficients of determination (R^2).

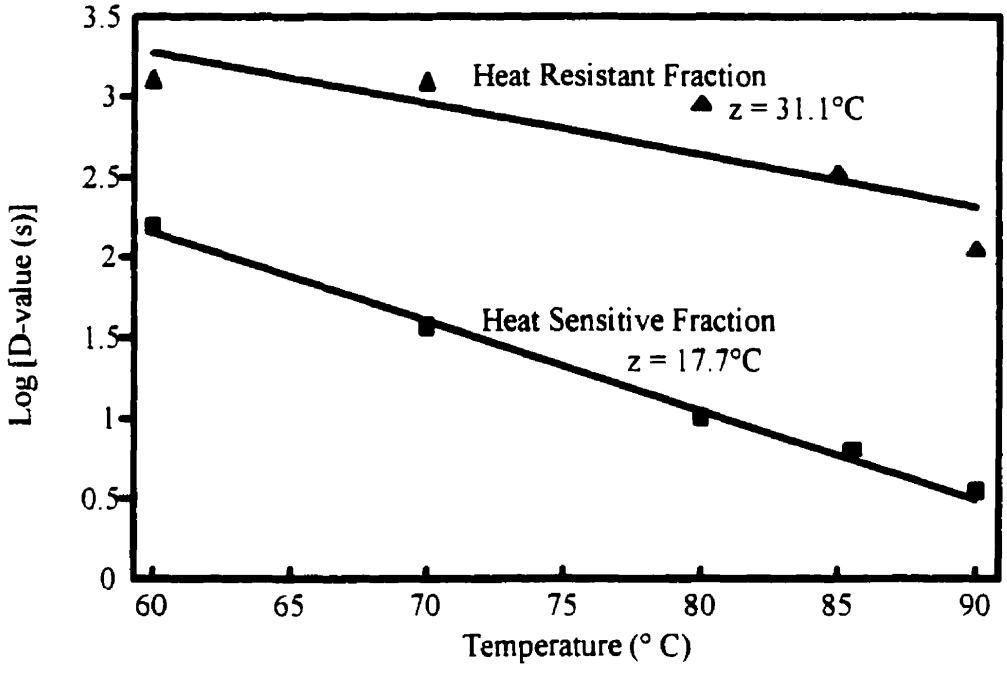

Figure 3.4 Temperature sensitivity curves of PME inactivation rates in orange juice (pH 3.7).

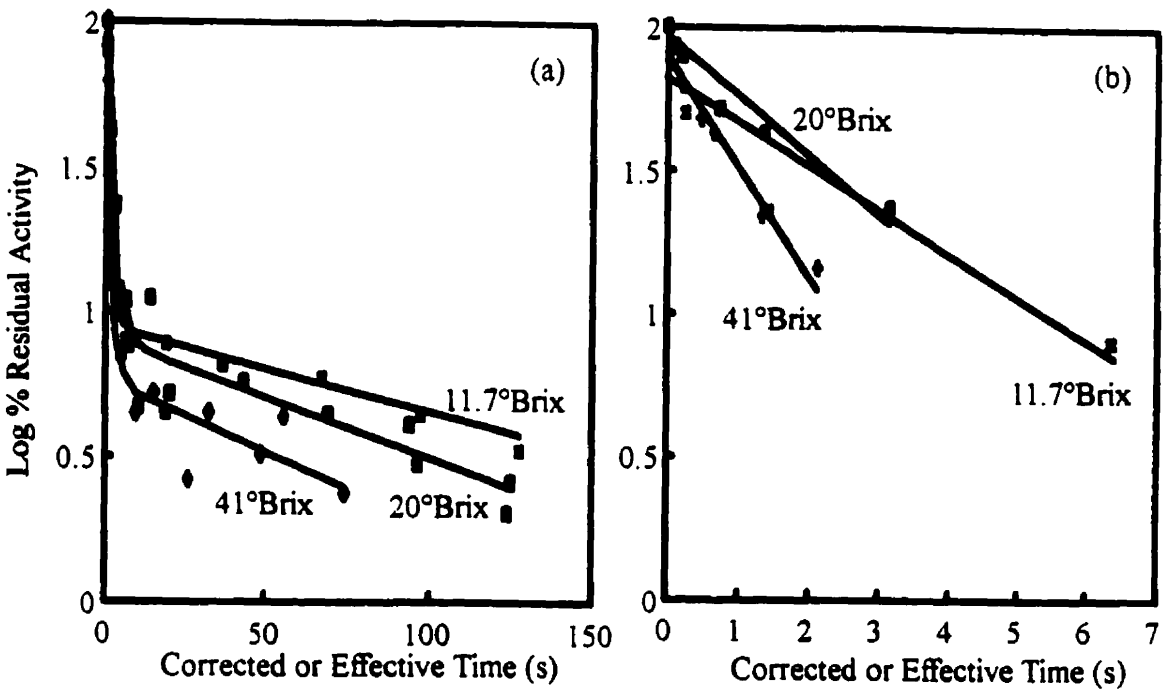

Figure 3.5 Thermal inactivation curves for PME in orange juice at various soluble solid concentrations showing (a) heat sensitive and heat resistant fractions and (b) an exploded view of inactivation of heat sensitive fraction.

The D-values were in the range 5-6 s for the single and double strength orange juice (11.7 - 20°Brix), while it was nearly 50% smaller for the 41°Brix concentrate indicating a faster inactivation rate to be associated with the latter. This could be due to the increased reactions of the PME with the increased amounts of pectin (Ogawa *et al.*, 1989). The differences associated with the heat resistant fractions were considerably smaller. Bissett *et al.* (1957) and Guyer *et al.* (1956) reported that juice with higher soluble solids content had no significant effect on PME inactivation. Some previous reports also demonstrated faster inactivation (lower D-value) at higher concentrations (Versteeg *et al.*, 1980; Bissett *et al.*, 1957; Carroll *et al.*, 1957), however, Marshall *et al.* (1985) showed higher resistance of PME to thermal inactivation at higher solids concentrations and reasoned that it was due to the protective effect caused by the soluble solids.

Figures 3.6 illustrates PME inactivation at different pH (3.2, 3.7, 4.2 and 4.6) in the single strength orange juice (11.7°Brix) while heating at 85°C. The results show that PME inactivation was highly pH dependent. The estimated D-values are summarized in Table 3.4.

Table 3.4. Corrected decimal reduction times of PME in orange juice at various pH, 85°C and 11.7°Brix.

pH	D-value (s)			
	Heat Sensitive		Heat Resistant	
3.2	0.324	(0.99)*	258	(0.82)
3.7	6.69	(0.88)	329	(0.96)
4.2	7.16	(0.96)	401	(0.82)
4.6	12.9	(0.97)	760	(0.84)

* The values in parentheses are the coefficients of determination (R^2).

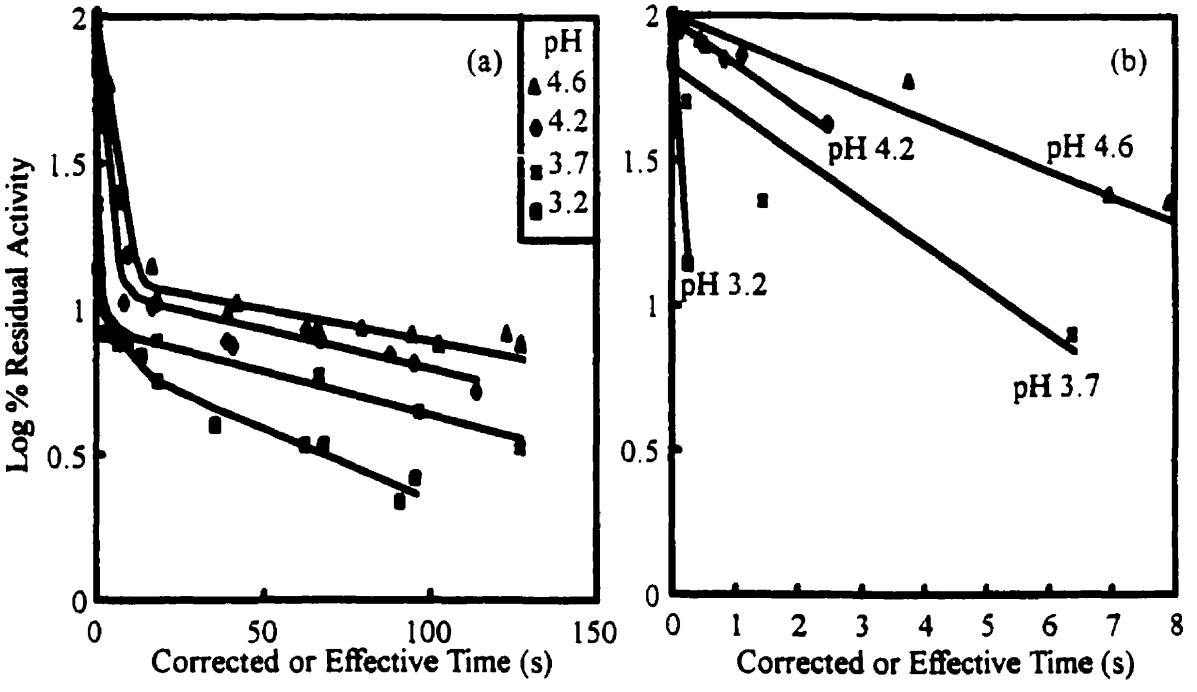

Figure 3.6 Thermal inactivation curves of PME in orange juice at various pH showing (a) heat sensitive and heat resistant fractions and (b) an exploded view of inactivation of heat sensitive fraction.

The enzyme PME was highly susceptible to thermal inactivation at lower pH unlike some other enzymes (like trypsin) which demonstrate increased thermal resistance at low pH levels (Awuah *et al.*, 1993). Similar findings have also been reported by others (Rouse and Atkins, 1952; 1953; Atkins and Rouse, 1954; Rothschild *et al.* (1975). Owusu-Yaw *et al.* (1988) reported that PME could be inactivated by employing low pH even without heat treatment. Again, Marshall *et al.* (1985) reported that between pH 4 and 7, PME was more sensitive to thermal inactivation at pH 7 than pH 4.

B. MICROBIAL DESTRUCTION IN APPLE JUICE

Come-up and come-down profiles and correction

As with PME inactivation studies, the time-temperature profiles of test samples at various temperatures essentially showed a logarithmic come-up profile. The come-up times ranged from 64 to 74 s. The heating times were corrected taking into the effective portion of come-up and come-down periods. The effectiveness increased with time up to CUT as summarized in Table 3.5 for test samples containing *S. cerevisiae* and *L. plantarum*. As with the PME inactivation, the contribution of CDT was negligible.

Thermal destruction kinetics

Determination of thermal resistance of microorganisms is usually achieved by exposing the cells to various heat treatments and evaluating the survivors. Traditionally, the microbial survival or destruction rate has been described by a first order rate resulting from the inactivation of a single site per cell (Charm, 1958; Stumbo, 1965). Figures 3.7 and 3.8 show typical survivor plots of *S. cerevisiae* and *L. plantarum* both before and after making lag corrections. As expected, the destruction behavior indicated a characteristic first-order rate kinetics. The destruction rates increased with increasing temperatures. It has been reported that the increase in destruction rates associated with higher temperatures could be explained by the increased number of water molecules possessing higher energy of inactivation and increased collision rate between surrounding water molecules and sensitive volumes within microbial cells (Charm, 1958).

Table 3.5. Heating conditions and CUT & CDT corrections for thermal inactivation of *S. cerevisiae* and *L. plantarum*.

Temp.	Average CUT	*S. cerevisiae*				*L. plantarum*			
		Uncorrected Heating time	Effectiveness of CUT	Corrections		Uncorrected Heating time	Effectiveness of CUT	Corrections	
(°C)	(s)	(s)	(%)	CUT (s)	CDT (s)	(s)	(%)	CUT (s)	CDT (s)
50	64	30	14	-26	+0.18	-	-	-	-
		60	42	-35	+0.32	-	-	-	-
		90-180	45	-35	+0.37	-	-	-	-
55	70	60	41	-35	+0.30	60	36	-38	+0.30
		90-120	48	-36	+0.38	120-240	43	-40	+0.38
60	70	15	2.1	-15	+0.09	30	7.8	-28	+0.15
		30	11	-27	+0.15	60	34	-40	+0.35
		45	26	-33	+0.20	-	-	-	
		60	39	-37	+0.35	-	-	-	
		75	46	-38	+0.38	75-120	42	-41	+0.38
70	72	10	0.66	-9.9	+0.05	10	0.23	-9.9	+0.05
		20	5.0	-19	+0.08	20	2.9	-19	+0.08
		30	12	-26	+0.15	30	7.9	-28	+0.15
		40	21	-32	+0.20	40	17	-33	+0.20
		-	-	-	-	50	27	-36	+0.25
		-	-	-	-	60	35	-39	+0.32
		-	-	-	-	70	43	-40	+0.35
80	74	-	-	-	-	15	0.01	-14	+0.05
		-	-	-	-	20	0.32	-19	+0.10
		-	-	-	-	30	1.8	-29	+0.15
		-	-	-	-	40	7.7	-0.7	+0.18

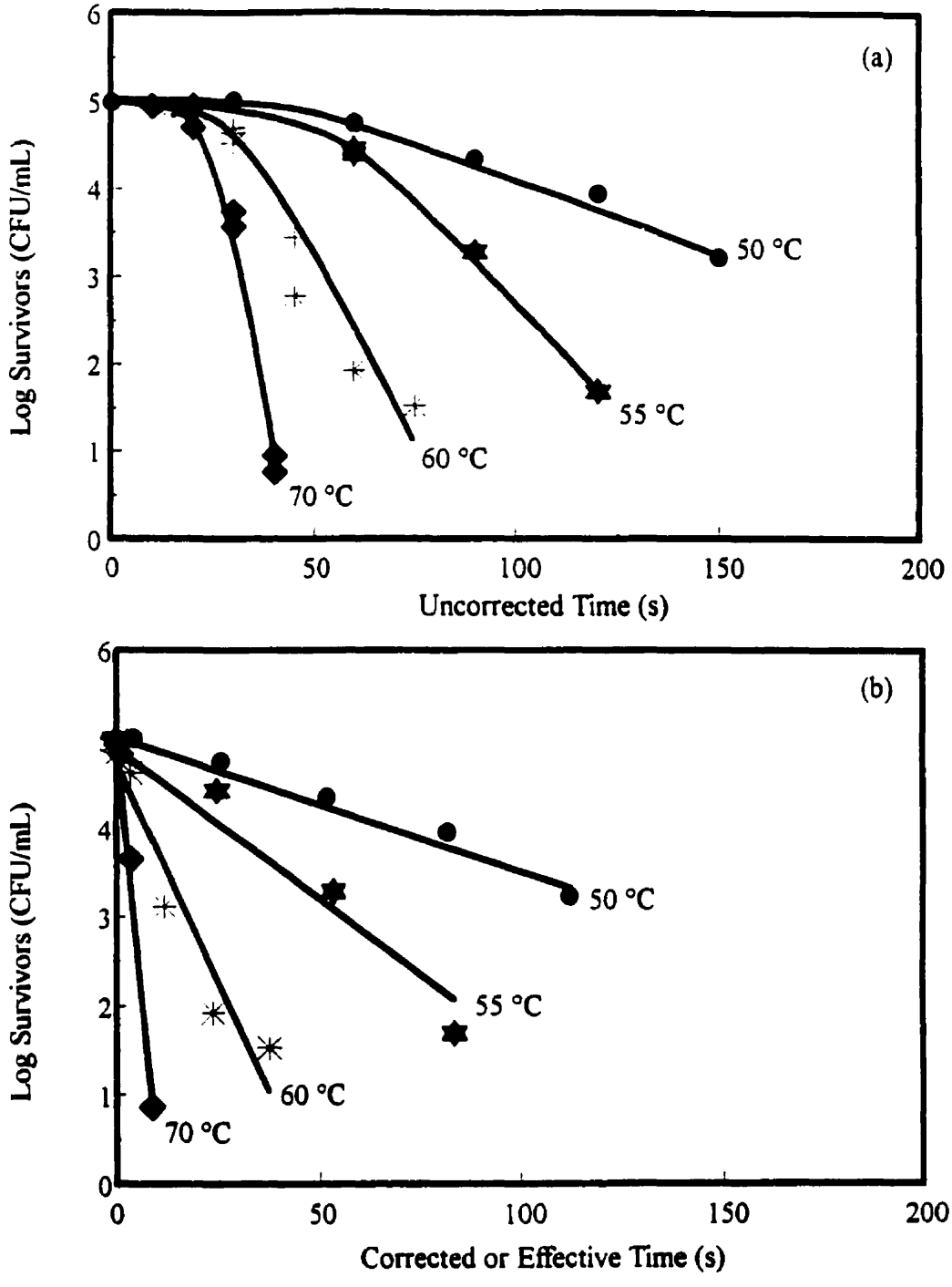

Figure 3.7 Survival curves of *S. cerevisiae* in apple juice (a) uncorrected and (b) corrected.

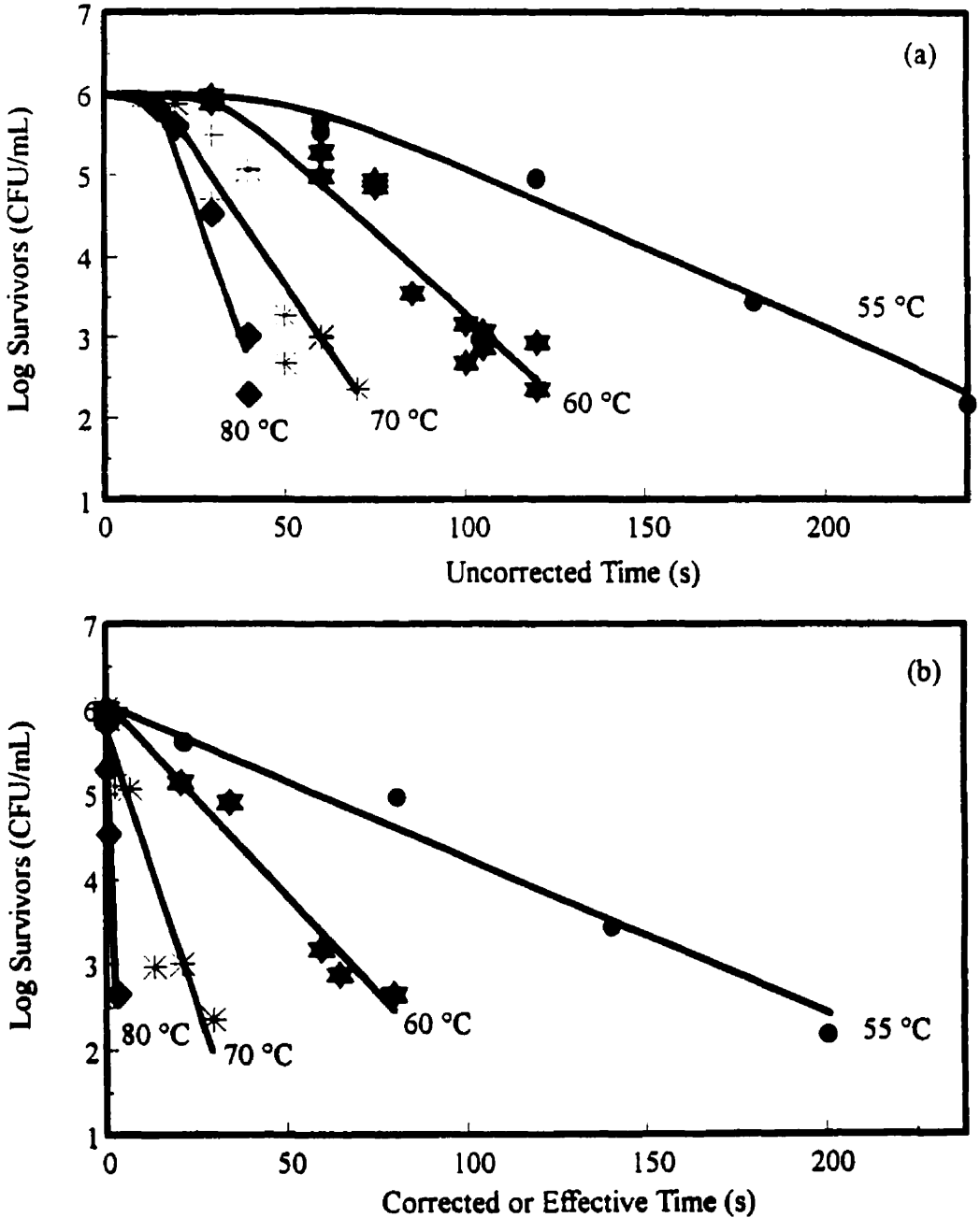

Figure 3.8 Survival curves of *L. plantarum* in apple juice (a) uncorrected and (b) corrected.

The destruction curves at various temperatures had a characteristic lag especially before making the corrections, the length of which decreased at higher temperatures. These lag periods (Figure 3.7a and 3.8a) were apparent since the temperatures did not reach the reference temperatures. The associated lag periods after the corrections (Figure 3.7b and 3.8b) were minimal as compared to the heating time. D-values ranged from 58 s at 50°C to 1.9 s at 70°C for *S. cerevisiae* and from 52 s at 55°C to 1.2 s at 80°C for *L. plantarum*. The results are summarized in Table 3.6. Figure 3.9 shows the temperature sensitivity of the inactivation rates (z-values) of the yeast and bacteria. The calculated z-values were 13.4 and 15.9°C for *S. cerevisiae* and *L. plantarum*, respectively.

Table 3.6. Thermal kinetic parameters (D and z-values) of spoilage yeast and bacteria in apple juice at various temperatures (pH 3.4).

Temp. (°C)	D-value (s)			
	S. cerevisiae		*L. plantarum*	
	Uncorrected	Corrected[1]	Uncorrected	Corrected[1]
50	60.1 (0.97)[2]	57.8 (0.98)	- -	- -
55	22.2 (0.99)	25.1 (0.97)	50.8 (0.98)	52.5 (0.98)
60	14.3 (0.94)	10.1 (0.93)	24.9 (0.94)	21.9 (0.97)
70	5.03 (0.95)	1.89 (0.99)	13.5 (0.91)	8.44 (0.87)
80	- -	- -	7.60 (0.94)	1.20 (0.98)
z (°C)	**19.4 (0.99)**	**13.4 (0.99)**	**31.9 (0.96)**	**15.9 (0.98)**

[1] CUT and CDT corrected D-values.
[2] The values in parentheses are the coefficients of determination (R^2).

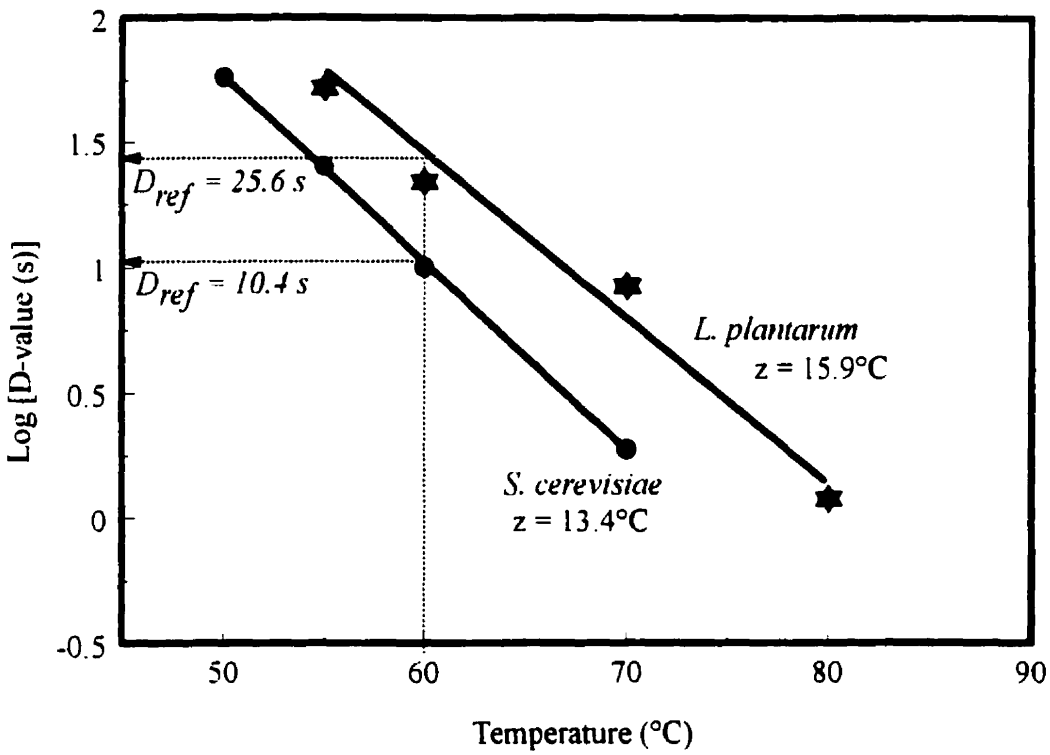

Figure 3.9 Temperature sensitivity curves for destruction rates of *S. cerevisiae* and *L. plantarum* in apple juice (pH 3.4).

Comparison of the thermal destruction of yeast and bacteria employed in this study showed some differences in the evaluated kinetic parameters (D and z-values). *S. cerevisiae* was more heat sensitive than *L. plantarum*, i.e., the D-values for *S. cerevisiae* were smaller. At an intermediate temperature of 60°C, the D-value of *S. cerevisiae* was about one half of that of *L. plantarum*. The associated z-value of *S. cerevisiae* was also lower than that of *L. plantarum* by a magnitude of about 15% indicating that its destruction was slightly more temperature sensitive than that of *L. plantarum*.

Comparison with literature values

A direct comparison of kinetic values from the present study with literature values may not be ideal due to difference in those inherent variables including age and strain of test culture, different batches of medium, pH, water activity, mode of destruction and type of juices (Török and King Jr., 1991). For *S. cerevisiae* in apple juice (8.6°Brix), Splittstoesser *et al.* (1986) reported D_{55} = 54 s (25 s from this study). Parish (1991) found D_{53} = 21-31 s (35 s in apple juice from this study) and 110-115 s (70 s in apple juice from this study) for *S. cerevisiae* and *L. plantarum* in grapefruit serum (17°Brix), respectively. Shomer *et al.* (1994) determined D_{60} = 4.1 s (10 s in apple juice from this study) for *S. cerevisiae* in orange juice obtained in capillaries and D_{58} = 7.8 s (15 s in apple juice from this study) obtained in a plate heat exchanger.

The z-values obtained for both microorganisms in the present study were higher as compared to those determined by Török and King Jr. (1991) [z (52-57°C) = 6°C for *S. cerevisiae* in spoiled apricots], Garza *et al.* (1994) [z (60-64°C) = 3°C for *S. cerevisiae* in spoiled peach puree] and Shomer *et al.* (1994) [z (50-60°C) = 5°C for *S. cerevisiae* in orange juice]. It should be noted that the literature values are not necessarily in the same range as in the present study. This discrepancy can be due to the fact that different strains of *S. cerevisiae* and *L. plantarum* in different medium were compared. In addition, range of temperature considered for obtaining z-values was different.

CONCLUSIONS

Thermal kinetics of enzyme inactivation and microbial destruction in fruit juices were investigated in the pasteurization temperature range. PME was selected for orange juice and *S. cerevisiae* and *L. plantarum* were selected as a spoilage yeast and a spoilage bacteria, respectively, for apple juice. The kinetic behaviors of enzyme inactivation microbial destruction could be well described by the traditional first-order reaction rate. Inactivation/destruction rates increased with an increase in temperature.

Two fractions of PME with different thermostabilities were found to exist. Taking effectiveness of CUT and CDT into consideration, kinetic parameters of PME in single-strength orange juice (11.7°Brix, pH 3.7) were characterized by reference D-values (at 70°C) of 39.3 and 914 s with z-values of 17.7°C and 31.1°C for heat sensitive and heat resistant fractions, respectively. The heat sensitive fraction accounted for approximately 92% of total activity. In addition, it was found that PME was more susceptible to thermal inactivation at lower pH and higher soluble solids concentration.

The two microorganisms in apple juice showed similarly to be easily inactivated; however, *S. cerevisiae* (ATCC 16664) appeared to be more heat sensitive than *L. plantarum* (ATCC 14917). With CUT and CDT contribution included, the reference D-values at 60°C of *S. cerevisiae* and *L. plantarum* were 10.4 s and 25.6 s with z-values of 13.4 and 15.9°C, respectively.

CHAPTER IV

CONTINUOUS-FLOW MICROWAVE HEATING: SET-UP EVALUATION AND USEFULNESS FOR KINETIC STUDIES

ABSTRACT

A microwave heating system was evaluated for subjecting liquid foods to continuous-flow heat-hold-cool pasteurization process. A domestic microwave oven (700 W, 2450 MHz) was modified to permit the continuous flow of liquids through a helical glass coil located centrally inside the oven cavity. For isothermal holding following microwave heating, the test liquid was passed through two helical coils submerged in a water bath preset to the exit temperature. The microwave heating system was calibrated to give the desired exit temperature as a function of fluid flow rate, heating volume and initial temperature under full-power microwave heating conditions. The average residence time of the fluid was computed by dividing the volumetric capacity of the helical tube inside the microwave oven by the volumetric flow rate under the test condition. Fluid temperature profile inside the heating coil was measured at specific locations using a fiberoptic temperature probe. By using calculated effective heating times, as detailed previously, and the residual PME activity obtained from test samples subjected to microwave heating and isothermal hold times, at 60°C, the inactivation rates were determined based on a first-order rate kinetics. Results indicated that the system could be used to study the inactivation kinetics under the microwave heating conditions as well as under isothermal hold, and that the D-values at 60°C under microwave heating conditions were considerably shorter than those obtained under conventional heating conditions.

INTRODUCTION

Thermal processing technology has come a long way since it was first introduced by Nicholas Appert in 1810 as an art of heat preservation. Traditional pasteurization of liquid foods such as fruit juices and beverages is routinely carried out under continuous high-temperature short-time (HTST) heating conditions using heat exchangers (tubular/plate) followed by a brief period of holding and subsequent cooling, again in heat exchangers, and packaging, usually under aseptic conditions. A drawback encountered in these continuous HTST pasteurization processes is the contact-surface fouling of heat exchanger (plate/tubular) caused by the exposure of fluids to high temperature surfaces.

Microwave heating can be an alternate thermal processing technique which prevents/minimizes surface fouling because of its ability to heat products internally in conjunction with its capability to penetrate deep which enables the fluid flowing inside microwave-transparent tubing to be heated without a significant differential increase in surface temperatures. Temperatures at contact surfaces are generally lower than the liquid bulk temperature due to heat exchange with relatively cooler surroundings. In addition, the process leads to rapid and uniform heating as compared to conventional pasteurization processes (Cole, 1993).

The use of microwaves for pasteurization of liquids in a continuous fashion has been investigated by Copson (1954). It was reported that PME inactivation in orange juice concentrate was achieved using a counter-flow system for exposing the juice concentrate to the microwave energy at 2450 MHz. The device consisted of double concentric glass bowls where the inlet fluid flowed in the outer bowl and overflowed into the inner bowl to the outlet. The apparatus allowed the sample to expose to microwaves from the top. Temperatures measured at the outlet were adjusted by varying magnetron anode current to achieve 54°C to 80°C. Mudgett (1980) studied performance models of milk for continuous-flow microwave pasteurization in a parallel-plate heat exchanger made of polystyrene. The sample was pumped at a constant flow rate through the heat exchanger and the outlet temperatures were controlled and adjusted by varying the voltage power. Kudra *et al.* (1991) employed a continuous-flow microwave heating system to study

heating characteristics of milk constituents. Test sample was pumped and circulated in a helical coil made of Tygon® tubing (165 mL) located centrally in a domestic microwave oven (700W, 2450 MHz). Temperatures were monitored at inlet and outlet ports outside the cavity. Nikdel and MacKellar (1992) similarly modified a microwave oven for a continuous flow of orange juice using Teflon tubing for heating and installing the holding and cooling sections at microwave exit. By varying the flow rates, various temperatures in a pasteurization temperature range (70 to 90°C) were obtained. Temperatures were only measured at the end of holding tubes and cooling coil. Recently, a pilot-scale continuous-flow microwave heating system was recently designed for pasteurization purpose (Nikdel and Burgener, 1993). Six domestic microwave ovens were utilized to increase temperatures of the products, which flow inside the tubing and being heating in the microwaves, in a step-wise manner from one oven to another.

In view of technological challenges associated with microwave heating, a detailed study on continuous pasteurization of orange juice was considered essential. The first step was to test if the desired temperatures could be achieved and subsequently to evaluate if microwave heating conditions would offer an advantage over conventional pasteurization with respect to enzyme inactivation/microbial destruction and quality improvement. The specific objectives of this phase of study were (1) to evaluate the heating characteristics of liquids in a continuous-flow microwave pasteurization system (for achieving desired exit temperatures as a function of the fluid flow rate) and (2) to test if the system could be used to evaluate PME inactivation kinetics in orange juice under continuous-flow microwave heating as well as isothermal holding.

MATERIALS AND METHODS

Microwave heating and isothermal holding system

A domestic microwave oven with a 700 W and 2450 MHz (Model RE-620TC, T. Eaton Co., Toronto, ON) was modified as detailed in Kudra *et al.* (1991) for continuous-flow microwave heating of liquids (Figure 4.1). The microwave oven used had cavity dimensions: 23 cm high, 35 cm wide and 33 cm long. The sample was run through a

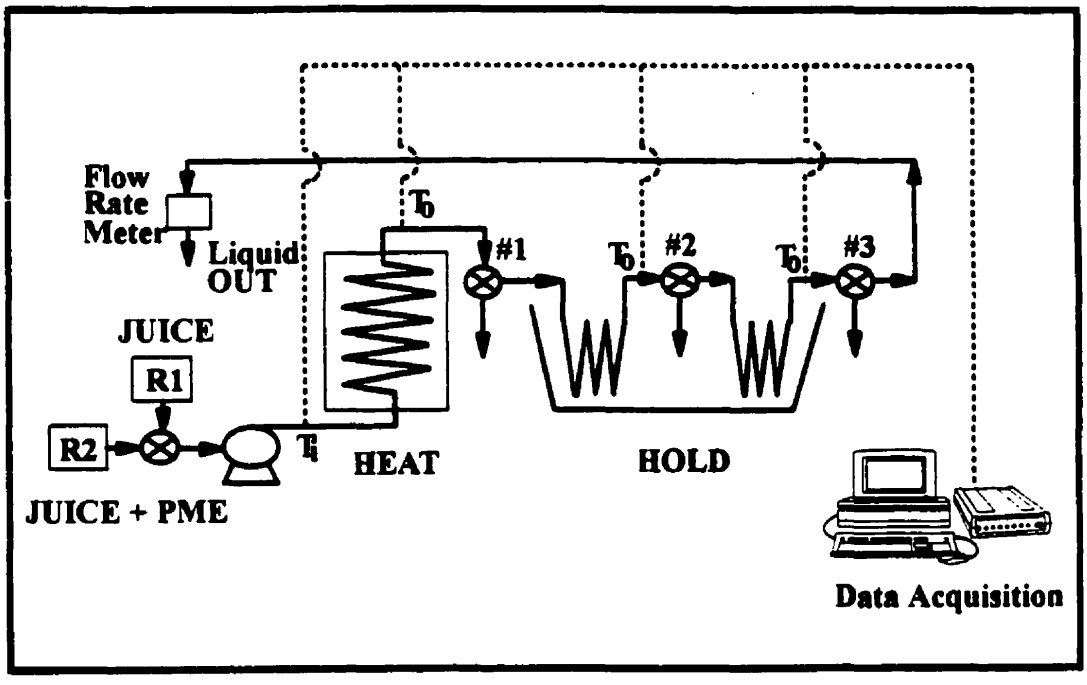

Figure 4.1 A schematic diagram of continuous-flow microwave heating and isothermal holding system set-up.

helical coil (125 mL capacity) made from Pyrex® glass tubing (9.6 mm i.d., 1.755 m length) centrally located within the microwave oven cavity. Inlet and outlet temperatures were monitored continuously using thin-wire (0.381 mm diameter) copper-constantan thermocouples (Omega Engineering Inc., Stamford, CT) positioned centrally within the tubing just outside the microwave oven. Temperature readings were recorded via a data-logger (Dash-8, Metra-Byte Corp., Taunton, MA). The juice to be run through the microwave was kept in a controlled temperature environment for achieving the desired initial temperature. The sample was pumped through the glass coil by a calibrated variable-speed metering pump (Monostat, New York, NY) and the inlet tubing outside the cavity was insulated to prevent sample warming.

For the initial evaluation of continuous-flow microwave heating characteristics, only the *heat* section of the system (Figure 4.1) was considered. The system was evaluated, first using water, for exit temperatures achieved at various flow rates and inlet temperatures. Orange juice (10°Brix) was later used and its heating characteristics were similarly determined. In addition to 125 mL heating capacity, a 250 mL heating coil was also employed for comparison.

In the heat-hold set-up, the test liquid exiting from the microwave oven was run through an isothermal holding system which consisted of Tygon® tubing (12.8 mm o.d., 9.6 mm i.d., and 1.685 m length) in a well agitated water bath (HAAKE, Fisher Scientific Ltd., Montreal, PQ). All tubings located outside the bath were insulated to prevent heat loss. The water bath was set to operate at the average outlet temperature of the fluid exiting from the microwave oven and was found to maintain the set-point temperature within $\pm$ 0.5°C. Three valves (#1, #2 and #3 in Figure 4.1) were installed along the exit line, one immediately outside the microwave oven and the other two at 0.855, and 1.685 m from the first (forming the two holding tube segments). Sample temperatures were recorded at all three locations along the holding tube. Test liquid was run through the system until a steady-state heating condition was established as indicated by a constant temperature at all three thermocouple locations. Test samples were withdrawn from these three-way valves: #1 gave samples of immediately following microwave treatment, #2 after holding in the first segment and #3 after holding in the first and second segments.

The treatment times were based on the average residence time of the juice in these three sections which in turn depended on the prevailing flow rates. In order to prevent flow disturbances in the system, test sample was drawn first from port #3 (downstream) followed by #2 and then from #1 (Figure 4.1), after steady-state conditions were established.

Orange juice preparation

The single strength orange juice (10°Brix, pH 3.7, 12% pulp), prepared by reconstituting commercial frozen concentrated orange juice with distilled water (1:3), was used for determining microwave heating characteristics. For the evaluation of enzyme inactivation kinetics, freshly squeezed orange juice was used instead of the reconstituted juice and prepared as detailed in the previous chapter. In some cases, fresh juice with naturally occurring PME was used thereby reducing the requirement of the expensive commercial enzyme since the natural and commercial PME showed similar inactivation kinetics [uncorrected D-values at 60°C were 195 s (natural) and 199 s (commerical), respectively for the same heating condition].

PME inactivation in orange juice

During the stabilization period, the reconstituted orange juice from reservoir R1 was circulated through the system. Following stabilization as outlined above, the three-way valve prior to the inlet port was switched to the reservoir (R2) containing the enzyme enriched (or fresh squeezed) orange juice (maintained at the same initial temperature). Test sample was run through the system enough long to purge out all the orange juice previously present in the tube and to reestablish the steady-state condition. Test samples were then withdrawn from the three ports, one at a time, and immediately cooled in containers in an ice-water bath and residual PME activities were determined.

For the purpose of verification of the set-up, microwave and the isothermal hold enzyme-inactivation kinetics presented in this study were limited to an exit temperature of 60°C. Initially, different initial temperatures and flow rates were employed to obtain different residence times. Subsequently, it was also found necessary to alter the tube

length (varied between 0.41 and 1.9 m) inside the microwave oven to accommodate different effective heating times. Low loss and high heat resistant plastic tubing (Norprene®, 12.8 mm o.d., 9.6 mm i.d.) was used for preparing coil lengths other than 1.755 m (125 mL).

Liquid time-temperature profile inside the microwave cavity

Time-temperature profiles of test samples during continuous-flow microwave heating were determined using a fiberoptic probe inserted centrally inside the tubing at three locations: one-fourth, middle and three-fourths of the heating tube length (1.19 m long, 85 mL). Temperature readings were recorded using Luxtron (model #755, Santa Clara, CA). Effective heating times (t_e) at the exit temperature were computed based on the evaluated time-temperature profile of the fluid (from inlet to outlet) and average fluid residence time as previously detailed in Chapter III.

Estimation of PME activity and data analysis

Subsequent to heat-cool and heat-hold-cool process, residual PME activities from treated samples were determined by pH titration method as previously detailed in Chapter III. Based on the first-order reaction kinetics, kinetic parameters (D-values) were evaluated from a plot of log (% residual activity) vs. heating times following microwave heat treatment at exit temperature of 60°C as well as those obtained following heat-hold treatment.

As the fluid flows through the helical coil in the microwave the temperature increases from an initial value, T_i, at the entrance port, to a final temperature, T_o, as it exits the microwave cavity. The duration for the temperature rise is the average residence time calculated by dividing the volume of test sample in the cavity by the volumetric flow rate. This time can be treated as equivalence of the traditional CUT of kinetic processes. The established way of accommodating the CUT during the continuous flow is to compute a corrected or effective heating time (t_e), based on the product time-temperature profile during the come-up period, which will be equivalent to holding the product at a reference temperature (Ball, 1923; Nath and Ranganna, 1977abc; Awuah *et al.*, 1993).

This is done by integrating the thermal effects of the time-temperature profile under consideration using Eqn.(3.5) as detailed earlier in Chapter III. A flow chart of the procedure for calculating accumulative lethality (F) or effective heating time (t_e) and kinetic parameters (D and z-values) was given in Figure 3.2. It should be noted that the kinetic data handling in the set-up described above gives the kinetic parameters under microwave heating conditions which results from a combination of thermal and any additional microwave effects, if existed. The z-value was obtained from previously established conventional thermal kinetics (Chapter III, z = 17.7°C). Since kinetic evaluation in this study was carried out only to verify the suitability of the set-up and as observed in the previous chapter, the CDT contribution to the PME inactivation was considered relatively small and neglected in the computation of D-values.

RESULTS AND DISCUSSION

Microwave heating characteristics

Exit temperature profiles for both water and orange juice (Figure 4.2a and 4.2b) obtained by pumping liquid through the microwave heating system at different rates showed typical lag periods prior to achieving steady state. The nonlinearity in time-temperature data during early phase of heating (the lag period) can easily be explained by the heat sink contributed by the coil and environment within the cavity as explained by Kudra *et al.* (1991). Temperature profiles observed for the exit temperatures during heating of both water and orange juice were similar. The equilibrium exit temperatures reached depended on the prevailing flow rates for both water and orange juice. Figures 4.3a and 4.3b show the sample temperature rise for water and orange juice as a function of flow rate, essentially showing a linear relationship. Lower flow rates resulted in a higher temperature rise and, hence, higher exit temperatures. The temperature rise was related to the average residence time of the fluid in the heating coil. Small deviations from linearity were observed at very low flow rates (long residence times) with exit temperatures exceeding 90°C due to the occasional boiling of liquid within the tube. These conditions were excluded from consideration. Some studies were also carried out with two

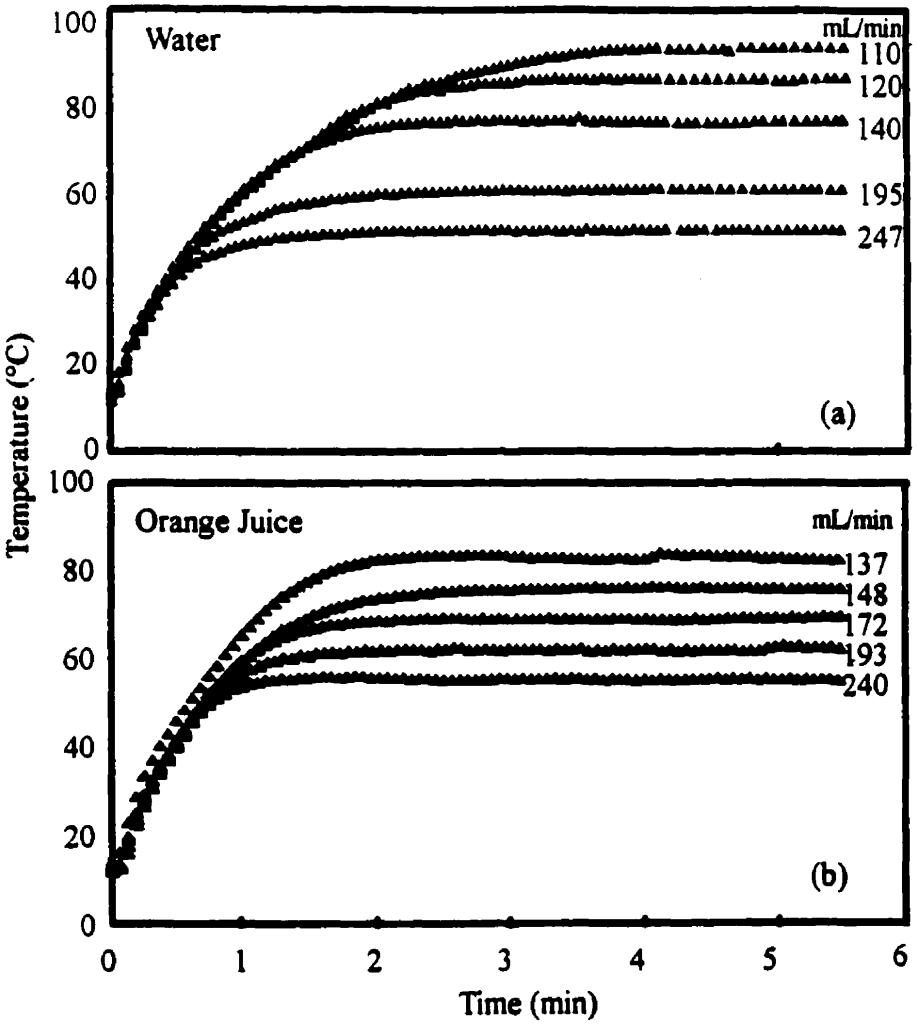

Figure 4.2 Microwave heating curves for (a) water and (b) orange juice as a function of flow rate.

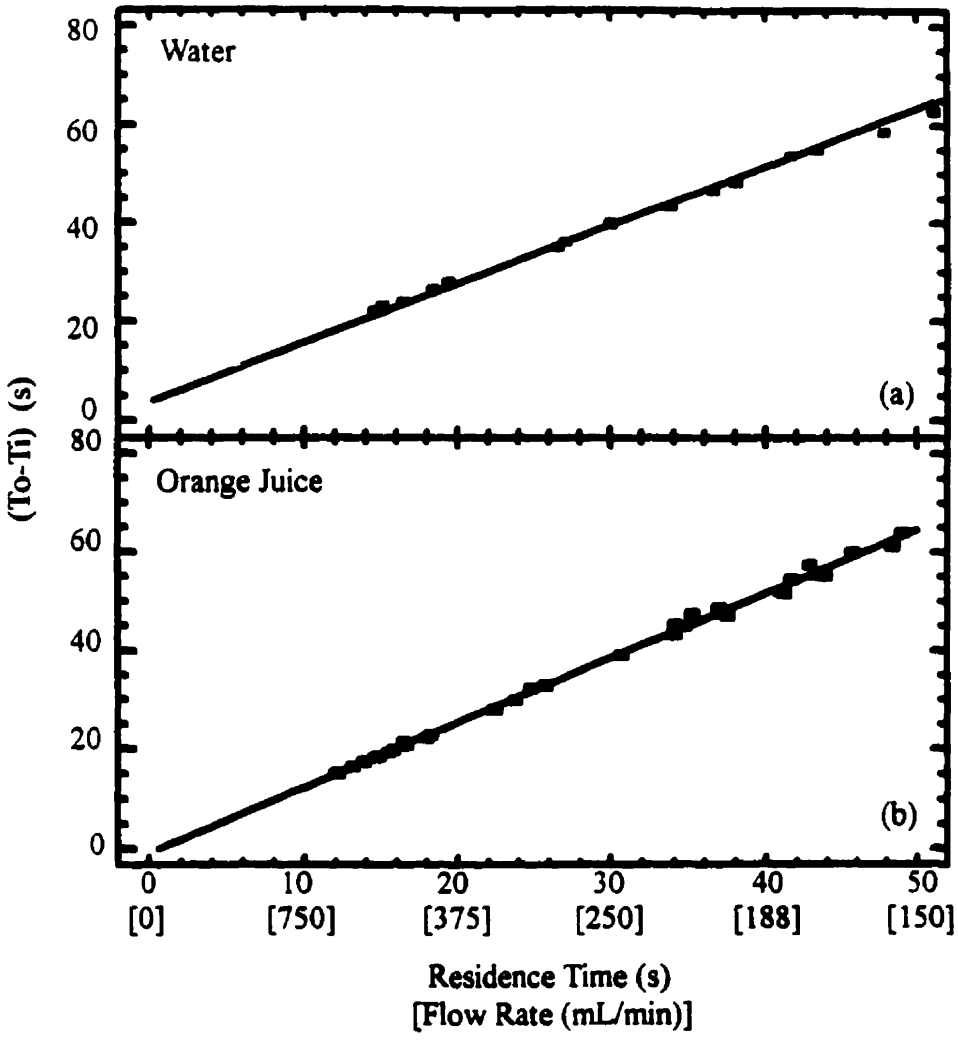

Figure 4.3 Temperature rise for (a) water and (b) orange juice as a function of residence time [flow rate] (125 mL heating volume).

coils (250 mL heating volume) placed inside the cavity oven. The relationship between temperature rise was again linear (Figure 4.4), however, the magnitude of temperature rise at any given flow rate was approximately one half as compared with the similar run one coil. Simple regression of data yielded R^2 value exceeding 0.95. The heating rate [R = (T_o-T_i)/time] for water in the 125 mL coil was 1.268°C/s and that for the orange juice was slightly higher, 1.284°C/s. With the 250 mL coil, as can be expected, the heating rate for orange juice was approximately one half of that for 125 mL coil: 0.675°C/s.

Temperature stability in the microwave pasteurization system

Temperature stability in the microwave pasteurization system with isothermal holding is illustrated in Figure 4.5. The figure demonstrates temperatures at the three exit ports #1 to #3 as a function of heating time from the time microwave oven was turned on. The outlet temperatures generally stabilized after 5-6 min at which time (indicated by an S in the figure) the inlet was switched from R1 (reconstituted orange juice) to R2 (fresh squeezed or enzyme enriched orange juice) which caused a small disturbance of the system. Again, as indicated in the figure, after an additional 3-4 min stability was again achieved and the fluid temperatures from the microwave exit to the holding tube exit remained stable. Samples were generally drawn only after the stability was achieved (10-12 min).

Time-temperature profiles during microwave heating

Dimensionless temperatures $[(T_o-T)/(T_o-T_i)]$ at the inlet, outlet and at the three locations (25, 50 and 75% of total length) [T_i, $T_{1/4}$, $T_{1/2}$, $T_{3/4}$, T_o, respectively] during microwave heating are summarized in Table 4.1. For a fluid flowing through the tube at a given flow rate, a representative time-temperature profile as a function of the residence time fraction can easily be obtained from the above data as shown in Figure 4.6. The results showed that temperature rise was somewhat non-linear with the temperature registered generally higher than in a linearly increasing profile. The temperatures can be generalized at any given length for the same diameter of tube as follows:

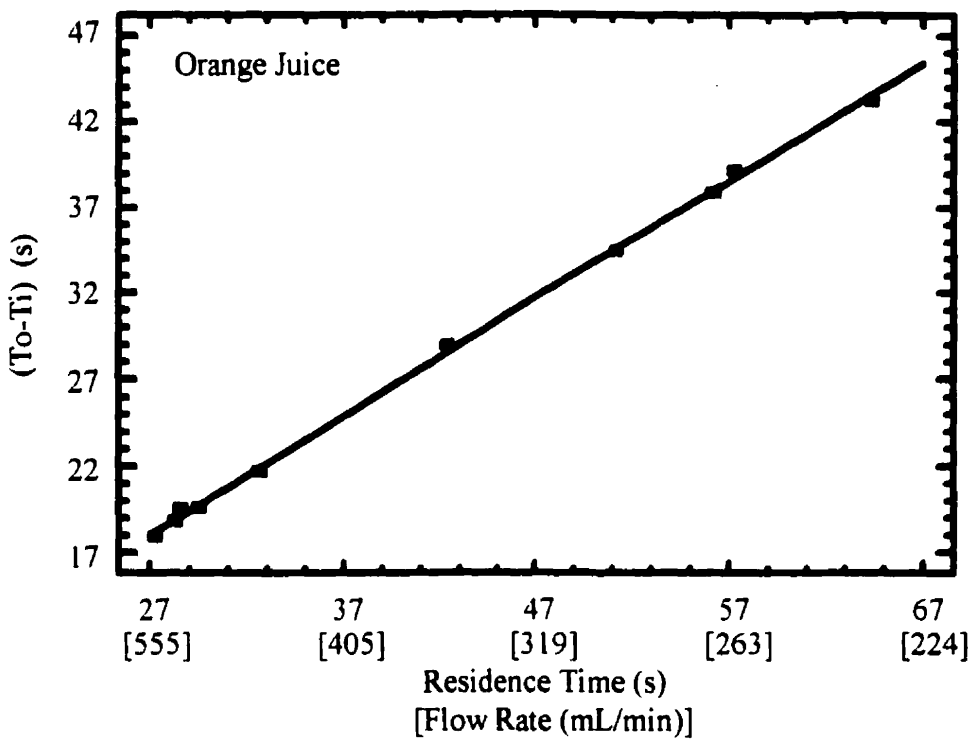

Figure 4.4 Temperature rise for orange juice as a function of residence time [flow rate] (250 mL heating volume).

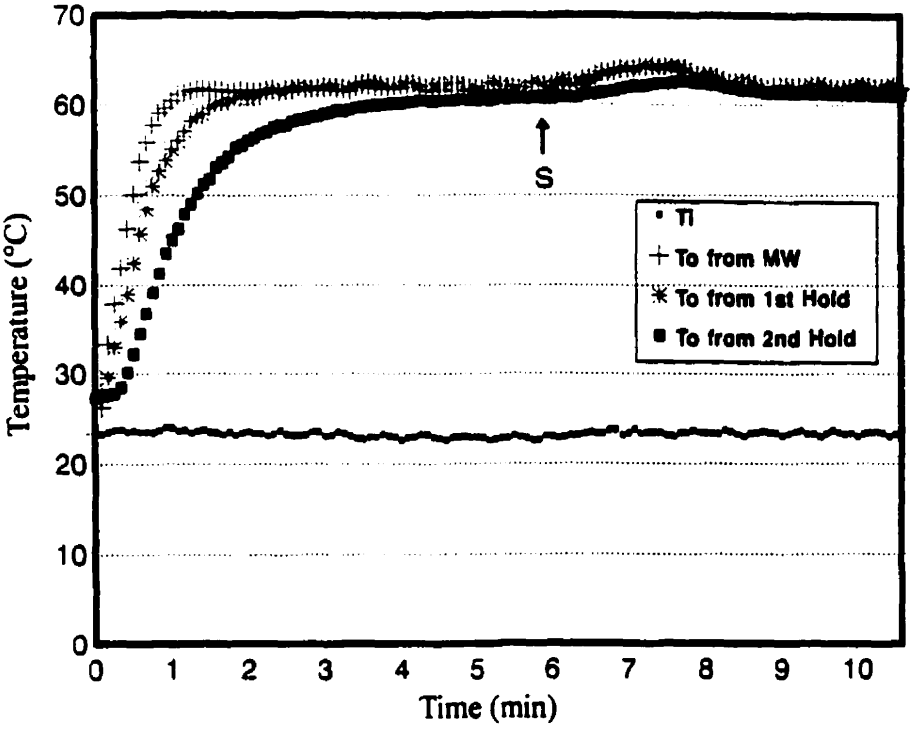

Figure 4.5 Temperature of orange juice during continuous-flow microwave heating and isothermal holding at 251 mL/min (125 mL heating volume).

At 25 % of total residence time; $T_{1/4} = T_o - [(T_0-T_i) \times 0.640)]$ (4.4)

At 50 % of total residence time; $T_{1/2} = T_o - [(T_0-T_i) \times 0.333)]$ (4.5)

At 75 % of total residence time; $T_{3/4} = T_o - [(T_0-T_i) \times 0.097)]$ (4.6)

Table 4.1 Dimensionless temperatures of orange juice during continuous-flow microwave heating at various thermocouple locations.

Position	$(T_o-T)/(T_o-T_i) \pm$ S.D.
T_i	1
$T_{1/4}$	0.640 ± 0.015
$T_{1/2}$	0.333 ± 0.025
$T_{3/4}$	0.097 ± 0.027
T_o	0

T_i , $T_{1/4}$,$T_{1/2}$,$T_{3/4}$ and T_o are temperatures at entrance port, one-fourth, middle, three-fourth and exit port positions along the heating tube length.

Inactivation kinetics following microwave heating

As with the thermal inactivation kinetics (Chapter III), the effective microwave heating times (t_e) represent the effective portion of the total residence time of the liquid heated in the microwave oven. The effective microwave heating times were computed based on the time-temperature profile for each test condition (shown typically in Figure 4.6) using Eqn.(3.5). The required z-value (17.7°C) was obtained from the thermal inactivation kinetics data (Table 3.2). The effectiveness (or effective percentage of the residence time) ranged from 28-33% depending on the flow rate (or residence time) and initial temperature of the fluid (Table 4.2). It should be noted that the kinetic analysis in

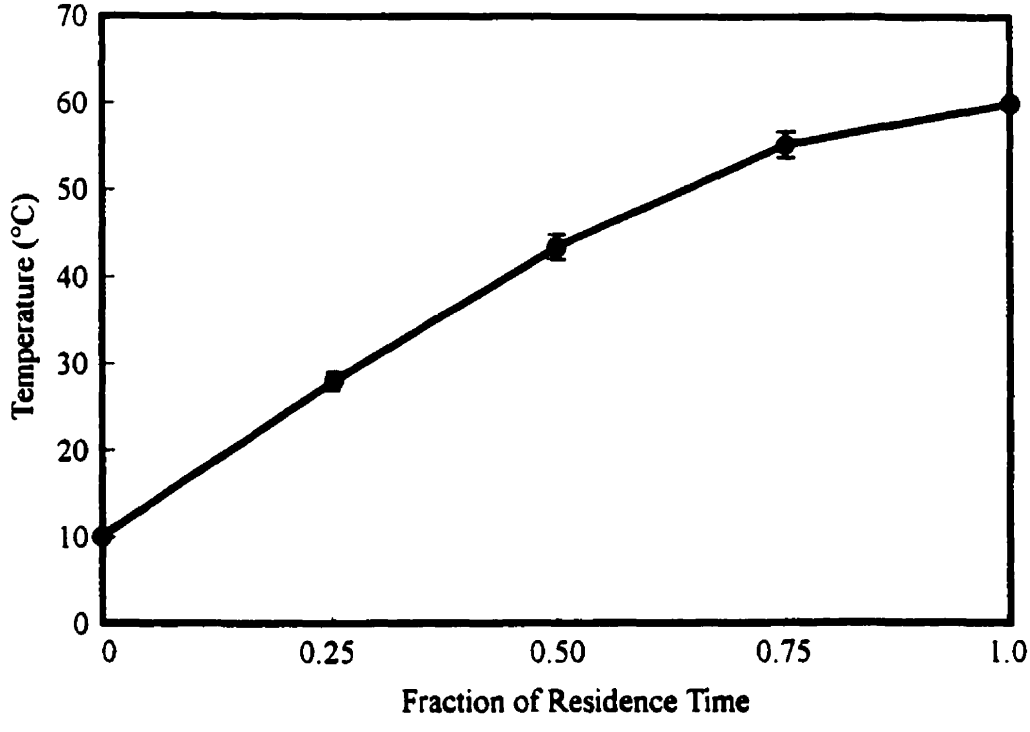

Figure 4.6 Time-temperature profile of orange juice during continuous-flow microwave heating as a function of residence time fraction.

Table 4.2. Heating conditions for continuous-flow microwave inactivation of PME in orange juice at exit temperature of 60°C.

Tube Volume (cm^3)		Ti (°C)	Flow Rate (mL/min)	Residence Time (s)	Effectiveness (%)	EffectiveTime (s)
29.5	(0.41)[1]	14.4	173	10.2	28	2.84
57	(0.80)	13.3	197	17.4	28	4.86
85	(1.19)	11.8	198	25.8	28	7.21
125	(1.75)	10.8	200	37.5	28	10.3[2]
		12.9	203	36.9	28	10.5[2]
		23.3	251	29.9	33	9.86
135	(1.90)	12.0	198	40.9	28	11.4

[1] The values in parentheses are tube length (m).
[2] The average of these values were taken for the calculation of D-value.

this study only aimed at the verification of the continuous-flow system whether it is suitable for kinetic study. Since only one temperature was used, the z-value could not be corrected as shown in Figure 3.1. More detailed analysis of the kinetic behavior are given in later chapters.

Originally, it was thought that one could study the microwave inactivation kinetics of PME at a given exit temperature by varying the residence time for the fluid since it should be possible to get the same exit temperature by different combinations of flow rate and inlet temperatures. As shown in Table 4.2 at 125 mL, however, the effectiveness of heating simultaneously increased with the flow rate (or decreased with residence time). In other words, the longer the residence time taken to achieve a given exit temperature,

the lesser is the effectiveness of the residence time. The net effect was that, although the residence times under the different flow rates were different, they all gave approximately the same effective heating time. Alternate procedures employing different combination of flow rate and tube length were explored to get different effective heating times at selected exit temperatures such that more data could be used for computing the inactivation kinetics.

Enzyme enriched orange juice subjected to microwave heating under conditions outlined in Table 4.2 were evaluated for residual enzyme activities at the microwave exit. The decrease in activity between the initial and at the cavity exit is a measure of the microwave inactivation. The extent of inactivation (logarithm of % residual activity) was plotted against the effective heating time to demonstrate the semi-logarithmic (first-order) microwave inactivation curve (Figure 4.7). The actual D-value was obtained from the regression of logarithm of residual activity vs. effective heating time as the negative reciprocal slope. Figure 4.7 compares the rate of PME inactivation due to continuous-flow microwave heating with the previously reported conventional thermal inactivation (Data from Table 3.2 at 60 and 70°C). As illustrated by the steepness of the curves, the microwave inactivation rate was considerably higher than the thermal inactivation rate at the corresponding temperature (D-value ~ 22 s from microwaves compared with D-value of ~ 150 s from conventional inactivation).

In order to be certain that the observed higher inactivation under microwave heating conditions is not an artifact arising from inappropriate use of temperature for kinetic purposes, the radial temperature distribution at the microwave exit port was evaluated. The observed radial temperature differences were within a range of 3°C with the center-line temperature generally giving the lowest reading. When the thermocouples measuring the exit temperatures are located along the tube radius, it is therefore possible that the average temperature of the fluid be 1 to 2°C higher than observed. The smaller radial temperature gradients, found in this study at the low flow rates as employed, was probably due to the use of the helical coil which generally promotes secondary flow contributing to mixing of the fluids along the path. The residence times were also based on a volumetric average basis which might also have contributed to some errors.

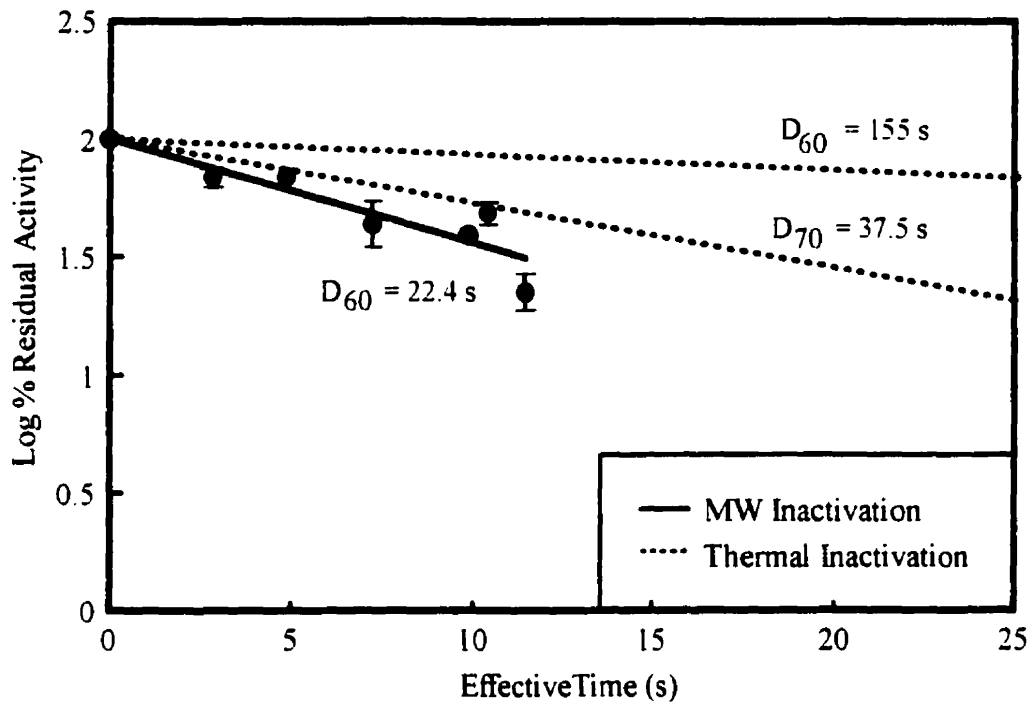

Figure 4.7 Semi-logarithmic plots of residual PME activity vs. effective heating time: microwave vs. thermal inactivation at pH 3.7.

However, as can be seen from Figure 4.7, the microwave inactivation rate at 60°C was even better than the thermal inactivation at 70°C, which would indicate a higher rate under microwave heating mode even if errors up to 10°C in temperature measurement existed.

Inactivation kinetics following microwave heating and isothermal holding

The change in PME activity between the two segments of the holding tube in the microwave set-up could be a measure of the thermal inactivation under continuous-flow conditions similar to the microwave set-up (Figure 4.8). The D-value calculated from this isothermal holding treatment was 103 s, still over four times higher than under microwave heating. The D-value calculated under isothermal heating conditions was also about 30% lower than the D-value determined previously under conventional batch heating conditions. The higher inactivation rate (lower D-value) in the continuous isothermal holding set-up as compared with the conventional batch mode thermal inactivation suggested that (1) there may be some residual effect from the microwave heat treatment causing the inactivation in the isothermal holding tubes to be better than in conventional heating or (2) it may be due to the inherent variations in the continuous set-up as discussed earlier with respect to radial temperature profiles and residence time distribution.

CONCLUSIONS

A continuous-flow microwave heating system was evaluated for carrying out kinetic studies under microwave heating characteristics of the liquids. The liquid was circulated through a section of heating tube centrally located in a microwave oven and a section of holding tube fully submerged in an adjacent well-stirred water bath operated at the microwave exit temperature. Under continuous-flow microwave heating conditions, exit temperatures of liquids were found to be a function of product flow rate, tube length (internal volumetric capacity) and initial temperature. The heating rate increased with decreased internal volumes. Constant exit temperatures of the fluid could be obtained

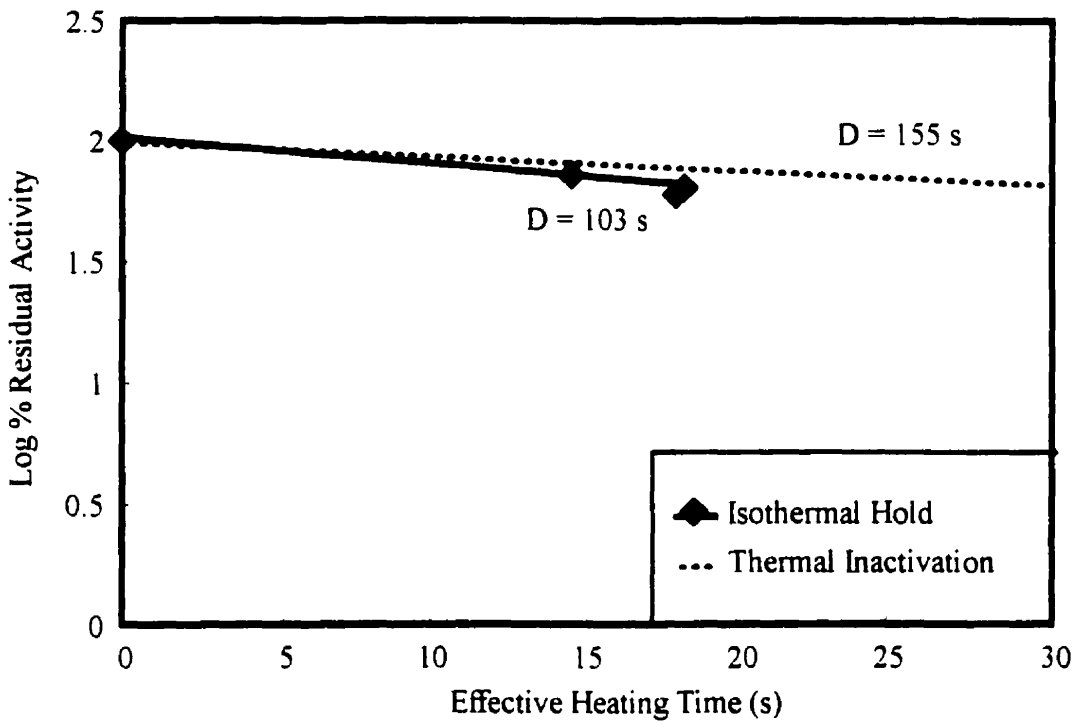

Figure 4.8 Semi-logarithmic plots of residual PME activity vs. effective heating time: isothermal holding vs. conventional heating at pH 3.7.

upon achieving steady-state condition. The system could be used for studying microwave inactivation kinetics by employing different combinations of tube length and flow rate to achieve various residence times and exit temperatures. Combinations of different flow rates and initial temperatures, however, resulted in the same effective heating time. Comparison of continuous-flow microwave and thermal inactivation kinetics of PME at 60°C indicated faster microwave inactivation rate as compared to conventional thermal inactivation rate.

CHAPTER V

KINETICS OF ENZYME INACTIVATION AND MICROBIAL DESTRUCTION UNDER CONTINUOUS-FLOW MICROWAVE HEATING CONDITIONS

ABSTRACT

Kinetics of enzyme inactivation in orange juice and microbial destruction in apple juice during continuous-flow microwave heating conditions were evaluated. PME enriched orange juice and inoculated apple juice were subjected to microwave heating in the system, described in the previous chapter, for various residence times to achieve selected exit temperatures (52.5 to 70°C). The residence times of the juice in the microwave oven, and the exit temperatures were dependent on the juice flow rate and length of the helical coil. The effective portions of come-up and come-down times were included in kinetic data analyses based on time-temperature profiles obtained during the heating and cooling. Results from the enzyme inactivation studies indicated that the time-corrected D-values of PME under microwave heating conditions were ~ 38, 12, 4.0 and 1.3 s at 55, 60, 65 and 70°C, respectively, as compared with ~ 150 and 37 s at 60 and 70°C under conventional heating, respectively. Results from the microbial destruction studies showed that the time corrected D-values ranged from 4.7 to 0.38 s for *S. cerevisiae* between 52.5 and 60°C, and from 14 to 0.33 s for *L. plantarum* between 57.5 to 65°C with corresponding z-values of 7.0 to 4.5°C, respectively. Initial lag phase of *S. cerevisiae* was apparent at all temperatures whereas for *L. plantarum* it was non-existent. Microwave inactivation of enzyme and destruction of microorganisms were found to be much faster than in conventional thermal mode at any given temperature. These results suggested some contributory non-thermal effects to be associated with microwave heating.

INTRODUCTION

The greater penetration depth and faster heating rates associated with microwave heating have been recognized as potential factors to improve the retention of thermolabile constituents in the fluid, e.g., milk, fruit juice (Mudgett, 1986). A number of studies carried out with liquid foods have reported successful microwave pasteurization of milk (Hamid *et al.* 1969; Jaynes 1975; Chiu *et al.* 1984; Merin and Rosenthal 1984; Knutson *et al.* 1988; Kudra *et al.* 1991). Microwave pasteurization of fruits and fruit juices, e.g., citrus juices, involving enzyme inactivation and microbial destruction, however, has not been commonly studied. Nevertheless, Copson (1954) reported that complete PME inactivation in orange juice concentrate was achieved at 66°C (580 W). Nikdel and MacKellar (1992) and Nikdel *et al.* (1993) studied the use of a continuous-flow microwave system to pasteurize orange juice using PME inactivation and bacterial count as indices and reported that > 99% inactivation was achieved with < 20 CFU/mL of *L. plantarum* at temperatures of 70 to 90°C for residence times of 15 to 25 s. The time to achieve the microwave exit temperature was considered to be negligible, CUT and CDT corrections were thus not applied in these studies. Therefore, only the holding times were considered. The inactivations of PME and *L. plantarum* were reported to be more pronounced using microwaves as compared to conventional heating. Recently, Abd El-Al *et al.* (1994) investigated inactivation of several enzymes including PME, polyphenol oxidase and peroxidase, in fruit juices, concentrates and pulps. They found that microwaves significantly reduced process times. However, no kinetic data on PME inactivation has been reported or time-corrected inactivation rates under microwave heating mode compared with the conventional thermal mode.

In order to establish a processing schedule for pasteurization of fruit juices such as orange and apple juices, kinetics of PME inactivation and microbial destruction are required. In Chapter IV, a technique was developed for evaluating the microwave inactivation kinetics of PME in orange juice. The emphasis, however, was especially on establishing the technique, and only one temperature condition was used for validating the technique rather than carrying out a complete analysis of inactivation kinetics.

The objectives of this study was, therefore, to evaluate the kinetic parameters (D-values and z-value) for inactivation of PME in orange juice and destruction of *S. cerevisiae* and *L. plantarum* in apple juice during continuous-flow non-isothermal microwave heating conditions by taking into consideration the temperature come-up and come-down contributions to lethality.

MATERIALS AND METHODS

A. ENZYME INACTIVATION IN ORANGE JUICE

Continuous-flow microwave heating

A 2450 MHz domestic microwave oven (Model RE-620TC, T.Eaton Co., Toronto, ON) with 700 W nominal power rating when operated at 100% full power was modified as detailed in Chapter IV to accommodate the continuous flow of orange juice at various flow rates. Since the objective was to evaluate only the microwave inactivation kinetics, the extra details related to the holding time were eliminated and the simplified set-up is shown in Figure 5.1a.

Fresh orange juice prepared as detailed in the previous chapters was run through the system until a steady-state heating condition was established as indicated by a steady exit temperature. Procedures used to achieve various exit temperatures (55, 60, 65 and 70°C) and accommodating different residence times for microwave enzyme-inactivation kinetic study are same as outlined in the previous chapter. The heat treated sample (~ 10 mL) was then withdrawn at the outlet port into a container submerged in an ice-water bath and residual PME activities were evaluated as detailed in Chapter III.

Measurement of time-temperature profiles

Time-temperature profiles of test samples during the continuous heating at various volumes were determined and equivalent heating times at the exit temperature were initially computed based on the predetermined time-temperature come-up profile and average residence time of the fluid as detailed in Chapter IV.

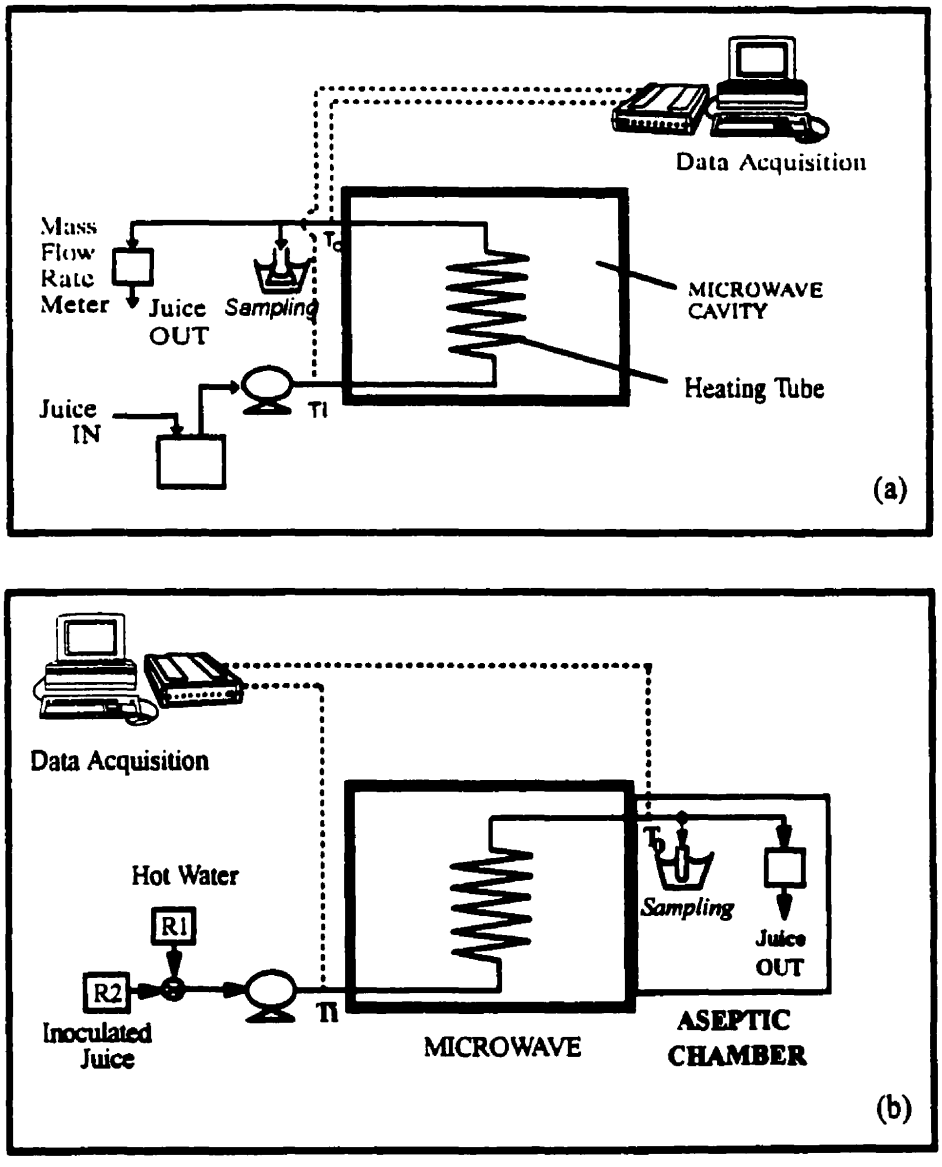

Figure 5.1 Schematic diagrams of continuous-flow microwave heating systems (a) enzyme inactivation in orange juice and (b) microbial destruction in apple juice.

In order to accommodate the cooling lag, time-temperature profiles of test samples were needed to be measured subsequent to their removal from the system. The test runs involved different flow rates (115 to 210 mL/min or about 2-4 mL/s) of orange juice. The approximately 10 mL test samples collected over a 3 to 5 s time-frame into precooled volumetric flasks (50 mL) submerged in an ice-water mixture were therefore expected to cool almost instantaneously. The CDT corrections at the 60°C experiments reported earlier in the previous chapter were small and hence were not applied initially. However, in order to make the analysis more complete and to test whether come-down corrections could be significant especially at higher temperatures, time-temperature profiles of 10 mL aliquotes of test samples, pre-heated to the exit temperatures, were recorded after transferring to a beaker surrounded by ice-water mixture, and the contributed lethalities were calculated using the previously reported (Chapter III) thermal kinetics data (because there is no microwave treatment during cooling).

Kinetic data handling: Non-isothermal microwave inactivation

Come-up period correction

In the majority of the previously described thermal inactivation procedures, the come-up period generally accounted only for a small portion of the overall heating time and therefore while finding out the effective heating times, the reference temperature is taken as the equilibrated medium temperature. The continuous-flow microwave heating set-up described earlier involves fully non-isothermal heating conditions with the temperature of the sample increasing continuously from the inlet to the exit. There is no isothermal hold period. Hence, the treatment of data is closer to that used in thermal processing situations. For this scenario, as before, first estimates of D-values (at exit temperatures) were calculated based on the total residence time of the product in the microwave cavity, and from D-values the first estimate of z-value was obtained. Correction of heating times and calculation of D and z were then be repeated to get convergence of values for D and z. It can be noted that since there is no specific isothermal hold period, the reference temperature used need not be the exit temperature. Any temperature within the range of study could be used as reference temperature. Thus,

each heating condition resulting in a certain inactivation of PME could be resolved to give equivalent heating times at each of the other selected temperatures under study. This helps in getting more data points at each temperature for getting more realistic D-values. The procedure, however, should followed only after the convergence of z-value using real data.

Come-down period correction

The early part of the cooling period could contribute significant lethality to the process especially if the cooling is slow. However, it should be noted that any contribution to inactivation of enzyme during the cooling period will only be due to thermal effects (since cooling is carried out outside the oven). The extent of thermal inactivation during cooling were computed for correcting the residual enzyme activities prior to kinetic data analyses. Using an approach similar to the one described earlier (Chapter III), the effective times during cooling, t_c, (i.e., as the equivalent time at the reference temperature) were computed from the cooling time-temperature curves as well as the z-value from thermal inactivation kinetics data (Table 3.2, Chapter III). The extent of thermal inactivation during the cooling was then calculated using the respective D-value as t_c/D (on the logarithmic scale). This value was added to the total residual activity to get the residual activity at the end of the heating period which was then used in the computation of D-values associated with microwave heating.

Sensory evaluation

Sensory evaluation was conducted to compare commercially available pasteurized orange juice before and after the microwave heat treatment. Fresh juice was not used in this case because it was not possible to reproduce commercial processing conditions for comparison purposes. The conventionally pasteurized juice with adjusted initial temperature of 20°C was run through a 57 mL heating tube in the microwave heating system at 160 mL/min (average microwave residence time of 21.4 s) resulting in 2.4 decimal reductions of PME.

A triangle test (Roessler *et al.*, 1978) was used for sensory evaluation in a fully-

equipped modern taste panel chamber in morning sessions. Test samples were prepared the day before, kept in a refrigerator and served cool. Samples (30 mL portion) were placed in amber glasses and coded with three-digit numbers. The order of the samples and the codes were randomized. A commercial software (The Compusense, Inc., Version 4.3, Guelph, ON) was used for design and analysis of the sensory evaluation data. The sensory evaluation was carried out under white lights and in silence. Panelists were presented with three samples and instructed to identify the odd sample for taste, flavor and odor. In addition, panelists were provided rinse water. The test was performed by a team of 30 panelists (age 20 to 40). Panelists were experienced with sensory evaluation procedures but were not specifically trained for these evaluations. The statistical table given by Roessler *et al.* (1978) was used for estimating significant difference at $p < 0.05$.

B. MICROBIAL DESTRUCTION IN APPLE JUICE

Apple juice preparation

Single strength apple juice was prepared according to the procedure detailed in Chapter III. The juice, inoculated with *S. cerevisiae* ATCC 16664 and *L. plantarum* ATCC 14917 (American Type Culture Collection (ATCC), Rockville, MD), was subjected to microwave heat treatments.

Continuous-flow microwave heating

Two stainless steel reserviors (20 L capacity) were used to feed the liquid to the system; one containing hot water (R1) for use during stabilization and the other containing inoculated apple juice (R2), with the three-way valve to switch the liquid from one tank to the other (Figure 5.1b). Flow rates were measured at the outlet. To prevent any contamination, the outlet or the sampling port was placed in an aseptic chamber which was pre-sanitized with liquid detergent to be free from microbes.

Testing procedures

Inoculated apple juice was prepared fresh each time to give an initial microbial

concentration of 10^5 - 10^6 CFU/mL for 20 L. All the tubings were cleaned by circulating hot water (65°C) through the system for 30 min before and after treatment. The cool juice was then run through the system long enough the purge out all the water previously present in the tube and to establish the steady-state condition indicated by a constant outlet temperature. The length of tubing and flow rate were pre-adjusted to obtain several heating times at each exit temperature. Test samples were withdrawn during the steady state heating conditions at exit temperatures of 45, 50, 52.5, 55, 57.5, 60, 62.5, 65 and 70°C at the outlet port in aseptic chamber. The treated sample (< 10 mL) was aseptically collected into a pre-cooled test tube and cooled immediately in ice-water bath. Subsequently, microbial survivors were enumerated as detailed in Chapter III. Based on first-order reaction rate, the kinetic parameters were analyzed by accommodating effective portion during CUT and contributory inactivation during CDT as detailed in the previous section. In addition, using a linear model of destruction, the duration of microbial lag phase (t_L), after applying the CUT and CDT corrections, was determined as a function of temperature and incorporated with the first-order model to describe the destruction behavior of the microorganisms (Kamau *et al.* 1990; Buchanan *et al.*, 1993). Sensory evaluation as detailed earlier was also performed with apple juice comparing conventionally pasteurized apple juice (from local market) with and without additional microwave treatment at 65°C to a level where complete destruction was achieved. The microwave treated sample was obtained by heating pasteurized apple juice (Ti = 15°C) through microwave heating unit (57 mL) at a flow rate of 170 mL/min (20 s residence time) giving a calculated 45 D process for *S. cerevisiae* and 11 D process for *L. plantarum*.

RESULTS AND DISCUSSION

A. ENZYME INACTIVATION IN ORANGE JUICE

Come-up and come-down period corrections

Figure 5.2 (heating section) shows a plot of the mean and standard deviations in

temperatures at three locations within the tube representing residence time fractions of 0.25, 0.5 and 0.75 in addition to the inlet (11°C) and outlet temperatures (60°C). The resulting plot again represented a slightly better temperature profile than for a linearly increasing temperature curve. The come-up time effectiveness was evaluated, employing the Fortran program (Appendix A) as the ratio of the effective time, t_e, at the exit temperature [based on Eqn. (3.5) after correcting D and z-values as detailed earlier] and total time assuming zero CUT (equal to the maximum residence time in this case). A cumulative effective heating time curve is also illustrated in Figure 5.2 giving a come-up period effectiveness of 5.2 s / 26 s or 20% for the heating period. The computed CUT effectiveness ranged from 15% to 22% depending upon the temperature and flow rate employed. The effective heating times represent the effective portion of the total residence time. These are compiled in the last column of Table 5.1 from which the D-values and z-values were subsequently obtained.

Figure 5.2 also shows the coupled cooling curve for the same condition and the calculated effective thermal time during cooling. As described earlier for CDT correction, PME inactivation during cooling was due to thermal contribution. At the first glance, it appears that significant inactivation could occur during the early period of cooling since the cooling time was in fact even longer than the heating time. However, there is a difference. The effective time during CDT accounts only for a small part of the long thermal time normally required for inactivation at the temperature specified. Hence, the extent of inactivation during cooling at different temperatures ranged from 3-10% of the total. For this example, the overall residual activity following both heating and cooling on the logarithmic scale was 1.636 (representing ~ 44% residual activity) which when combined with the effective microwave heating time of 5.2 s gives a single point D-value of 14.3 s. It can be noted that the effective time during cooling is 2.6 s at the exit temperature of 60°C. Based on the thermal D-value at 60°C of 155 s, the cooling period contribution to inactivation will be $[t_c/D]$ or 0.017 on the logarithmic scale (~ 1%). This activity when added to the total residual activity gives the heating period residual activity as 1.653 (or ~ 45%). Coupled with the effective heating time of 5.2 s, this will give a corrected D-value of 15.0 s, which is about 5% higher than the previously calculated 14.3

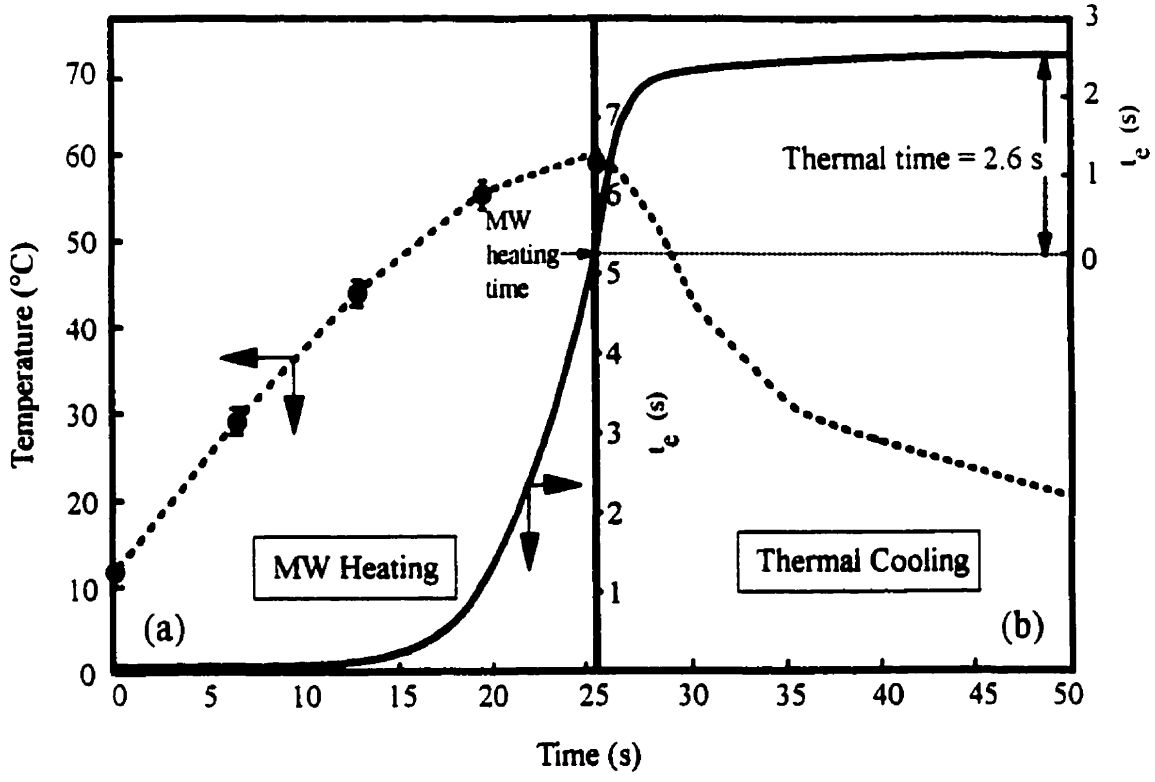

Figure 5.2 Time-temperature profiles during (a) microwave heating and (b) thermal cooling, and computed effective times.

Table 5.1. Heating conditions for continuous-flow microwave inactivation of PME.

Temp. (°C)	Volume (mL)	T_i (°C)	Flow Rate (mL/min)	Residence Time (s)	Effectiveness of CUT (%)	t_e (s)	t_c* (s)
55	57 (0.80)[1]	11.0	209	16.4	21.1	3.5	3.5
	85 (1.19)	11.8	211	24.2	21.8	5.3	3.5
60	15 (0.21)	11.3	157	5.7	18.5	1.1	2.6
	20 (0.28)	9.2	148	8.1	18.0	1.5	2.6
	29.5 (0.41)	14.4	173	10.2	19.2	1.9	2.6
	57 (0.80)	13.3	197	17.4	20.6	3.6	2.6
	85 (1.19)	11.8	198	25.8	20.3	5.2	2.6
	135 (1.90)	12.0	198	40.9	17.7	7.2	2.6
	250 (3.51)	13.2	206	72.8	19.7	14.3	2.6
		10.0	191	78.5	18.2	14.3	2.6
		11.4	194	77.3	20.1	15.5	2.6
65	15 (0.21)	12.5	139	6.5	17.2	1.1	2.0
	57 (0.80)	12.1	168	20.4	18.6	3.8	2.0
		9.7	161	21.2	18.0	3.8	2.0
	85 (1.19)	10.8	170	30.0	18.3	5.5	2.0
		13.0	170	30.0	17.2	5.2	2.0
70	15 (0.21)	11.4	123	7.3	16.9	1.2	1.6
		10.3	115	7.8	15.4	1.2	1.6

[1] The values in parenthesis are the heating tube length (m).

* Calculated based on thermal kinetics and used for corrections of residual PME activity.

s. It should be noted that the D-values reported in Table 5.2 are based on the regression of several data-pairs of log residual activities vs. effective heating times rather than the single point approach used in the above illustration. The corrected D-values were about 0.5-5% higher after applying correction to cooling period). As pointed out before these corrections are on the conservative side since the test sample under continuous-flow situation cooled much faster than the 10 mL sample used for temperature profile evaluation (especially at the higher temperatures which will have a lower flow rate).

Microwave inactivation kinetics

Microwave heating characteristics of orange juice were previously detailed in Chapter IV. Different lengths of heating tube were employed to get different effective heating times at the exit temperatures employed in the study (55, 60, 65, and 70°C). Enzyme inactivation under the conditions as indicated in Table 5.1 was evaluated for residual PME activities. The time-corrected enzyme activity as a function of heating time at various temperatures is shown in Figure 5.3. The curves demonstrated typical first-order inactivation kinetics showing linearity on a semi-logarithmic scale. The calculated D-values from the curves are summarized in Table 5.2, ranging from 38.5 s at 55°C to 1.32 s at 70°C with R^2 values of ~ 0.94. The results thus show higher inactivation rates at higher exit temperatures as indicated by the lower D-values. Temperature sensitivity of D-values of PME in orange juice during microwave heating is shown in Figure 5.4 indicating a z-value of 10.2°C (R^2 = 1.00).

It should be noted that the single D-value reported in the previous Chapter IV at 60°C was calculated based on the z-value of 17.7°C from thermal inactivation kinetics, which remained uncorrected since only one temperature was used. Using z-value of 10.2°C obtained in this chapter (more appropriate for microwave heating conditions), the recalculated D-value at 60°C for the condition described in Chapter IV would be 13.2 s which is within the same magnitude as observed in this study.

D-values obtained for the inactivation of PME in the conventional thermal mode as reported earlier in Chapter III were used for comparison. Versteeg *et al.* (1980) reported a D-value of 47 s at 60°C for PME with a z-value of 11°C under thermal mode.

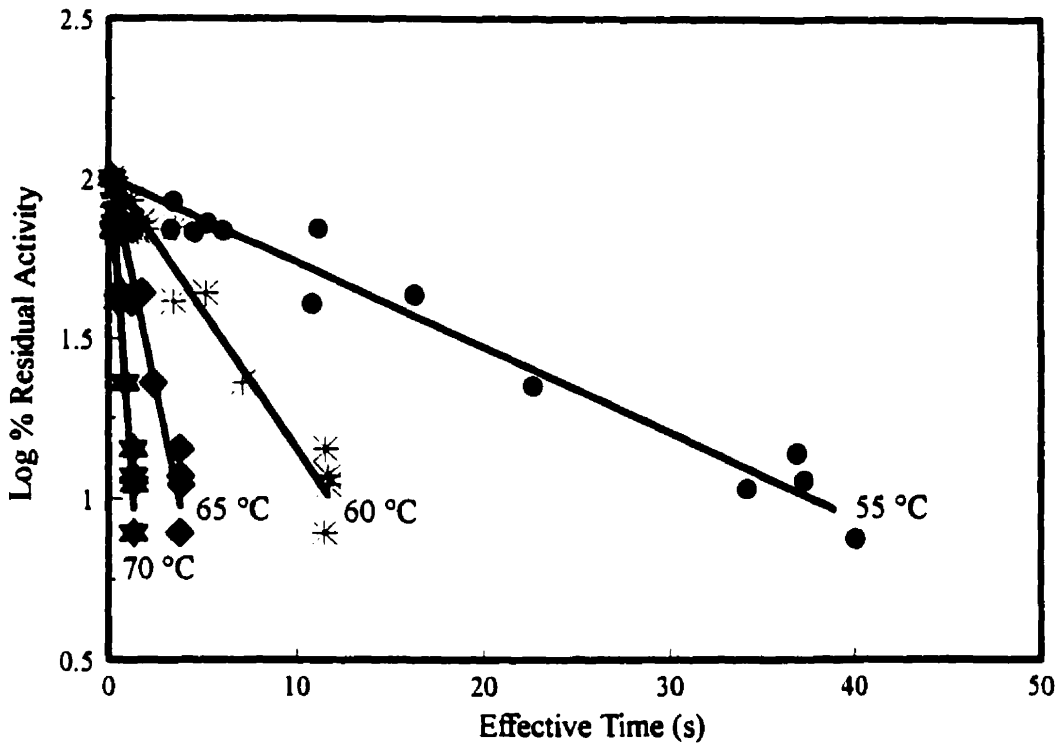

Figure 5.3 Semi-logarithmic plots of residual PME activity vs. effective heating time as a function of effective microwave heating times at various temperatures.

Table 5.2. Kinetic parameters of PME in orange juice (pH 3.7) determined by continuous-flow microwave inactivation.

Temperature (°C)	D-value (s)		
	Uncorrected	CUT corrected	CUT+CDT corrected
55	169	36.7	38.5 (0.94)[1]
60	68.0	12.0	12.4 (0.94)
65	25.6	3.97	3.98 (0.94)
70	7.54	1.31	1.32 (0.94)
z value (°C)	**11.2**	**10.4**	**10.2 (1.00)**

[1] The values in parentheses are the regression of determination (R^2).

Wicker and Temelli (1988) reported a D-value of 0.225 s at 90°C with a z-value of 10.8°C. The calculated D-values from these reports ranges from 130 to 390 s at 55°C and 6 to 36 s at 70°C. In comparison with these values, the D-values obtained under microwave heating conditions in the present study are smaller by an order of magnitude. This indicates that microwaves cause inactivation of PME in some way which cannot be solely explained by conventional thermal effects. In other words, these results confirm the contributory *non-thermal* effects of microwaves resulting in enhanced inactivation of PME in orange juice as initially observed in Chapter IV. There have been studies suggesting *non-thermal* effects on horseradish peroxidase, wheat germ lipase and soybean lipoxygenase enzymes (Henderson *et al.*, 1975; Kermasha *et al.*, 1993ab) and several microorganisms (Dreyfuss and Chipley, 1980; Khalil and Villota, 1988). Some possible mechanisms for the *non-thermal* effects of microwaves which will most likely be explained as structural changes in a microscopic and molecular level, have been detailed

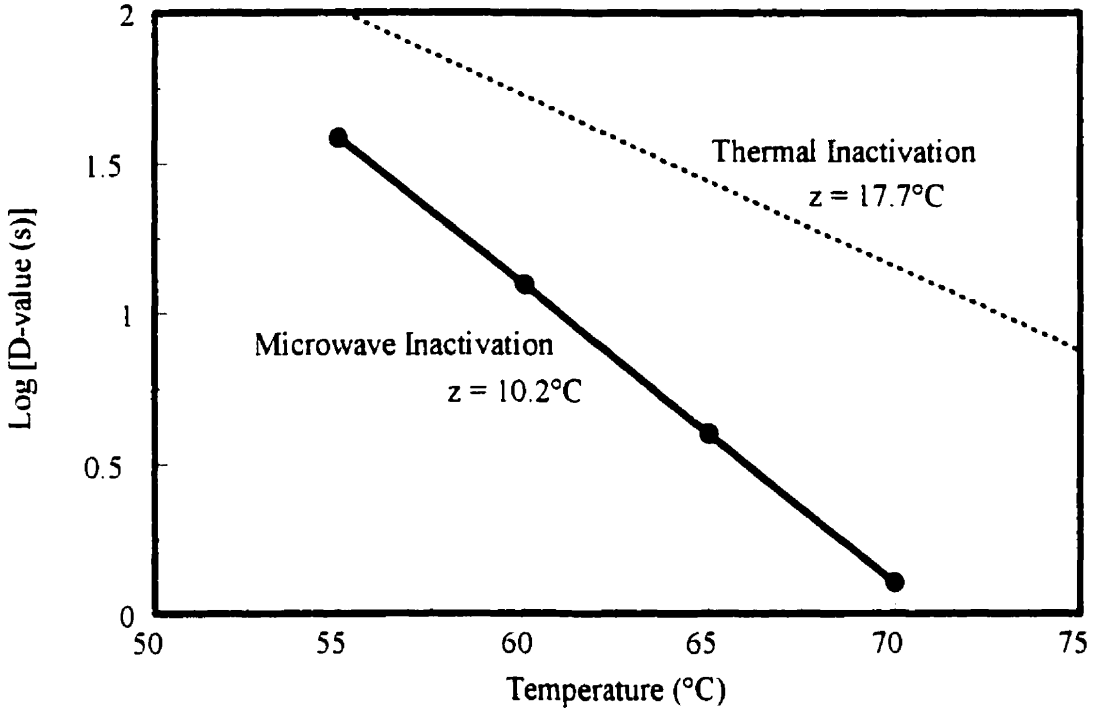

Figure 5.4 Temperature sensitivity curve of PME inactivation rates in orange juice (pH 3.4) during continuous-flow microwave heating.

in Olsen *et al.* (1966). However, there have also been reports (Goldblith and Wang, 1967; Goldblith *et al.*, 1968; Welt *et al.*, 1994) refuting the existence of *non-thermal* effects. These studies generally argue that the reported existence of *non-thermal* effects in literature have been a result of improper data handling or inadequate experimental set-up. A strong argument in favor of the non-existence of *non-thermal* effects is that the energy associated with microwaves is insufficient to disrupt covalent bonds. However, some non-covalent bonds such as hydrophobic, electrostatic and hydrogen bonds may be disrupted. In addition, enzymes, as protein complexes, which generally have numerous polar and charged moieties can easily be influenced by the electrical field of microwaves (von Hippel, 1954). A more thorough investigation and discussion addressing the issue of *non-thermal* effects are dealt within the next two chapters.

Sensory evaluation

The sensory evaluation was carried out to compare the conventionally pasteurized orange juice obtained from local market with that which received the additional microwave heat treatment at 70°C for an effective time of 3.2 s giving a 2.4 D process with respect to PME. Ten out of 30 panelists correctly identified the odd sample. The sensory evaluation results showed that the additional microwave treatment resulted in no statistically significant deviations ($p > 0.05$) in quality from the conventionally pasteurized orange juice. In addition, it showed that microwaves (for the given condition) did not adversely contribute to changes in the overall flavor profile.

It is probably considered unusual to compare the quality of conventionally pasteurized juice with similar samples subjected to additional microwave treatment for judging the influence of microwave treatment. Ideally, the comparison should have been microwave pasteurized products vs. its conventionally pasteurized counterpart. The former approach was used because it was not possible to subject the same fresh juice to both conventional pasteurization under commercial processing conditions and microwave pasteurization in the set-up being evaluated. Sample to sample differences would make the evaluation inappropriate if the test samples microwave pasteurized in the set-up were to be compared with commercial samples obtained from elsewhere. In the present

evaluation, the commercially processed juice served as the control to which microwave pasteurization treatment was given. The objective was to judge if the microwave treatment would result in adverse flavor changes and the result indicated no adverse effects.

B. MICROBIAL DESTRUCTION IN APPLE JUICE

Come-up and come-down period corrections

The time-temperature history of test samples during continuous-flow microwave heating were similar to those previously described (Figure 5.2) which were used for CUT and CDT effectiveness computation. The effectiveness ranged from 7.9 to 14% depending upon the sample size and temperature employed. Based on Eqn.(3.5) as previously described for kinetic data analyses, effective heating times were evaluated for each heating condition (Table 5.3) and used for obtaining CUT and CDT corrected D-values and z-values.

Microwave destruction kinetics

Inoculated apple juice was subjected to continuous-flow microwave heating. Sample volume and flow rate were adjusted to obtain desired exit temperatures. Initially, the microwave kinetics was carried out at 5°C temperature intervals from 45°C to 75°C. At $T \leq 50$ and 55°C, respectively, for *S. cerevisiae* and *L. plantarum*, viable counts per plate were too numerous. Furthermore, viable counts of > 30 CFU/mL per plate of *S. cerevisiae* and *L. plantarum* were possible only at temperatures up to 57.5°C and 65°C, respectively. Since the two selected strains were very sensitive to microwave heating, shorter temperature intervals were employed with various sizes of samples. A major problem of the microwave kinetics of microorganisms in this study was the selection of temperature since rapid destruction was achieved at temperatures as low as 60°C for *S. cerevisiae* and 65°C for *L. plantarum* which is 10-15°C lower than that used for thermal destruction. Furthermore, the maximum length of heating tubing that could be accommodated was 4.2 m which restricted the sample size in the oven to 300 mL. Therefore, kinetic parameters were evaluated at 52.5, 55, 57.5 and 60°C for *S. cerevisiae*

Table 5.3. Heating conditions for continuous-flow microwave destruction of *S. cerevisiae* and *L. plantarum* in apple juice.

Temp.	*S. cerevisiae*						*L. plantarum*					
(°C)	Volume (mL)	Flow Rate (mL/min)	Residence Time (s)	Effectiveness of CUT (%)	t_e (s)	t_e[4] (s)	Volume (mL)	Flow Rate (mL/min)	Residence Time (s)	Effectiveness of CUT (%)	t_e (s)	t_e[4] (s)
50	190[1] (2.66)[2]	275	41.4	-	-	-						
	250[1] (3.51)	255, 272	58.8, 55.1	-	-	-						
	300[1] (4.21)	310	58.1	-	-	-						
52.5	130 (1.82)	230	33.9	14.0	4.8	4.0						
	250 (3.51)	267	56.2	14.0	7.9	4.0						
	300 (4.21)	261	69.0	14.0	9.7	4.0						
55	130 (1.82)	256	30.5	13.3	4.0	3.5	130[1] (1.82)	256	30.5	-	-	-
	190 (2.66)	220	51.8	13.3	6.9	3.5	190[1] (2.66)	220	51.8	-	-	-
	250 (3.51)	235	63.8	13.3	8.5	3.5	250[1] (3.51)	235	63.8	-	-	-
	300 (4.21)	245	73.5	13.3	10	3.5	300[1] (4.21)	245	73.5	-	-	-
57.5	85 (1.19)	196	26.0	12.8	3.3	3.0	130 (1.82)	206,217	37.9,35.9	9.07	3.5,3.3	3.0
	130 (1.82)	217	35.9	12.8	4.6	3.0	300 (4.21)	213	84.5	9.07	7.7	3.0
	150[1] (2.11)	205	43.9	12.8	5.6	3.0						
	190[1] (2.66)	210	54.3	12.8	6.9	3.0						
60	70[3] (0.983)	215	19.5	12.3	2.4	2.6	85 (1.19)	190,200	26.8,25.5	8.66	2.3,2.2	2.6
							130 (1.82)	217,213	35.9,36.6	8.66	3.1,3.2	2.6
							190 (2.66)	198,200,	57.6,57.0	8.66	5.0,4.9	2.6
							250 (3.51)	215,210	69.8,71.4	8.66	6.0,6.2	2.6
							300 (4.21)	251,221,	71.7,81.4,	8.66	6.2,7.1	2.6
								225,205,	80.0,87.8,	8.66	6.9,7.6	2.6
								208	86.5	8.66	7.5	2.6
62.5							85 (1.19)	168,170	30.3,30	8.29	2.5,2.5	2.2
								180	28.3	8.29	2.4	2.2
65							57[3] (0.800)	170,174	20.1,19.6	7.95	1.6, 1.6	2.0

[1] The number of survivors were too numerous and not taken for kinetic data analyses, [2] The values in parentheses are the heating tube length (m), [3] Complete destruction was observed, [4] Calculated based on thermal kinetics and used for corrections of survivors.

and 57.5, 60, 62.5 and 65°C for *L. plantarum*.

Figure 5.5 and 5.6 illustrate microwave destruction kinetics of the two strains showing both uncorrected and corrected heating times. Results indicated characteristic first order reaction kinetics during microwave heating. The rate of destruction generally increased with an increase in temperature. The calculated D-values as summarized in Table 5.4 ranged from 4.75 s at 52.5°C to 0.378 s at 60°C for *S. cerevisiae* and from 14.1 s at 57.5°C to 0.327 s at 65°C for *L. plantarum*. At higher temperatures, enumeration was possible only with the first treatment time since longer heating time gave complete destruction. To obtain more precise D-values at these conditions, initial microbial lag phases were accommodated after applying CUT and CDT corrections. The lag phase was observed only with *S. cerevisiae*. The lag phase duration (t_L) of *S. cerevisiae* was determined as a function of temperature (Figure 5.5b) and the t_L at 60°C was predicted to be 0.86 s based on a linear regression (R^2=0.92). Accommodating the associated lag period, the D_{60} of *S. cerevisiae* was then predicted based on a two-point determination. The D-value obtained for this conditions was only an estimate at 0.378 s.

The significance of t_L is more apparent especially when considering a process time by utilizing the D-value. As suggested by Buchanan *et al.* (1993), the process lethality can be considered as follows:

$$F_T = t_L + (nD_T) \qquad (5.1)$$

where F is a process lethality at any given temperature, n is a number of decimal reduction desired and D is a D-value at the same temperature. In the thermal inactivation study detailed in Chapter III, t_L was not apparent because of its smaller magnitude in relation to the heating times employed (1-3 s compared with ~ 150 s). When the destruction time required became shorter as in the case of microwave heating, the lag periods became more apparent and significant.

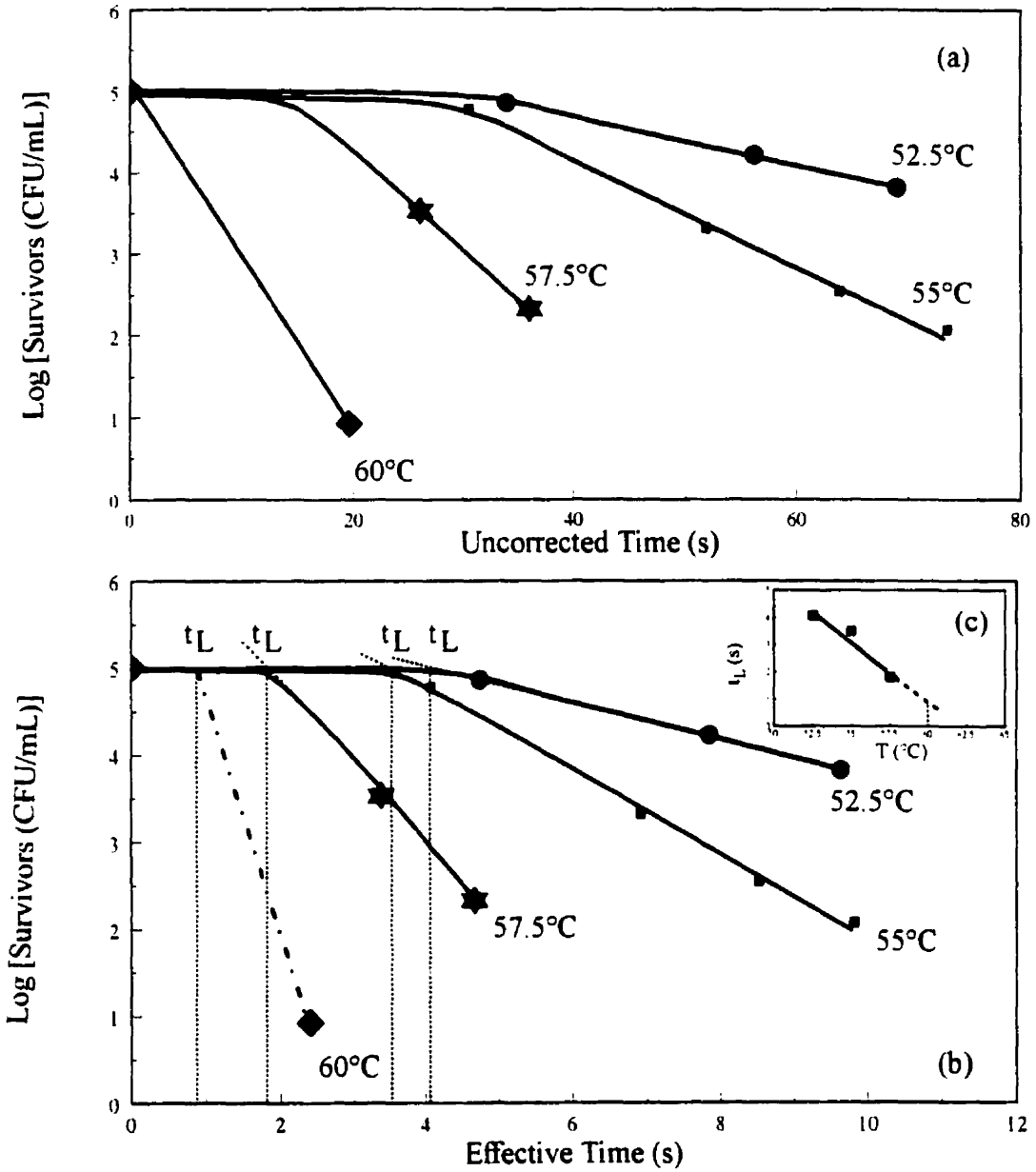

Figure 5.5 Survival curves of *S. cerevisiae* in apple juice during microwave heating as a function of (a) uncorrected and (b) corrected heating times [inset (c) shows the microbial lag phase as a function of temperature].

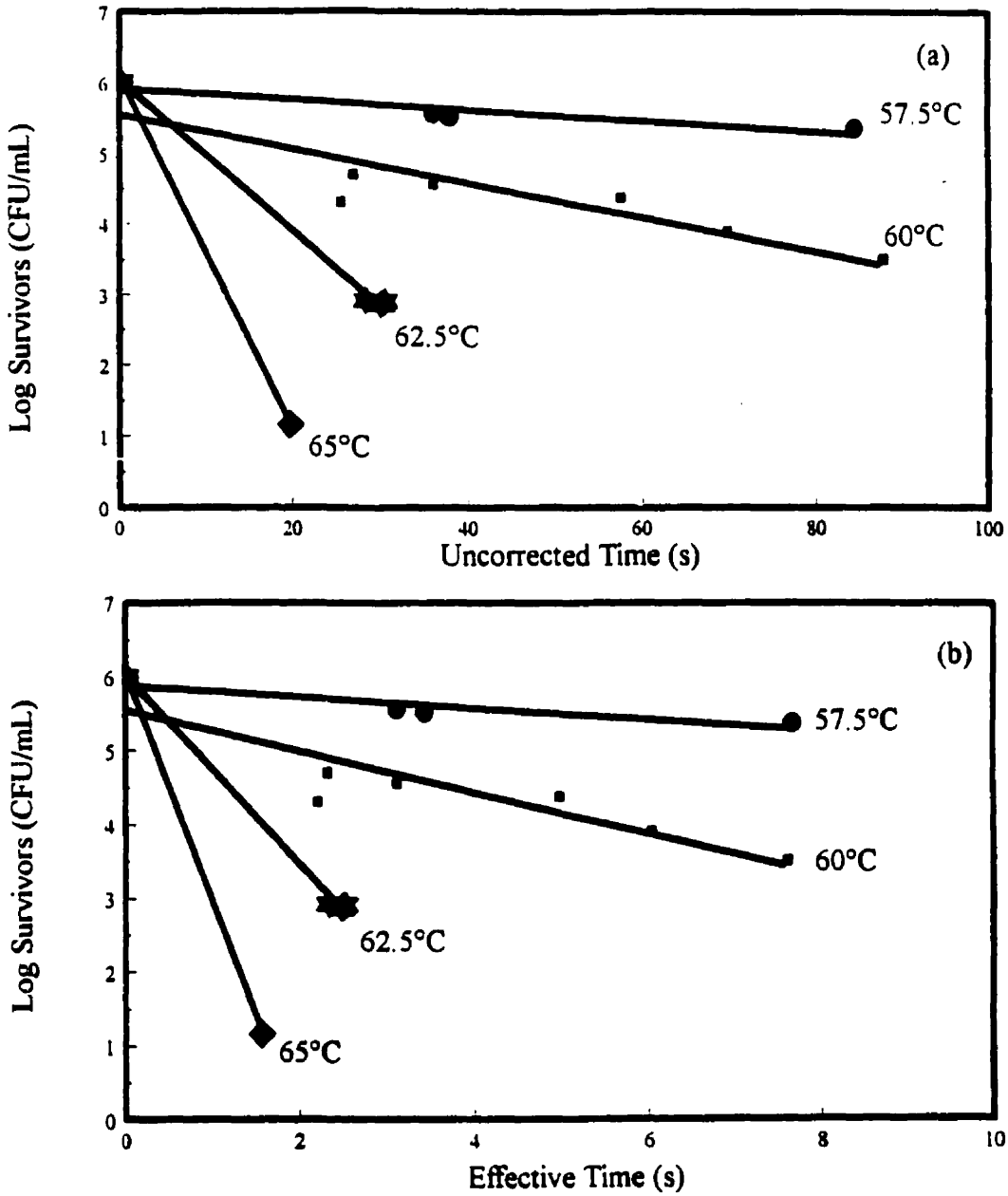

Figure 5.6 Survival curves of *L. plantarum* in apple juice during continuous-flow microwave heating as a function of (a) uncorrected and (b) corrected heating times.

Table 5.4. Kinetic parameters of spoilage microorganisms in apple juice (pH 3.4) during continuous-flow microwave heating.

Temp. (°C)	*S. cerevisiae*			*L. plantarum*		
	Uncorrected D-value (s)	Corrected[1] D-value (s)	t_L (s)	Uncorrected D-value (s)	Corrected[1] D-value (s)	t_L (s)
52.5	34.0 (0.94)[2]	4.75 (0.94)	4.1	-	-	-
55.0	15.6 (0.98)	2.08 (0.98)	3.5	-	-	-
57.5	8.25 (0.97)	1.07 (0.97)	1.8	154 (0.85)	14.1 (0.85)	0
60.0	4.79 (0.99)	0.378[3] -	0.86[3]	44.3 (0.82)	3.83 (0.82)	0
62.5	-	-		9.58 (0.99)	0.794 (0.99)	0
65.0	-	-		4.11 (0.99)	0.327 (0.99)	0
z (°C)	**8.83 (0.99)**	**6.97 (0.99)**		**4.64 (0.99)**	**4.48 (0.99)**	

[1] CUT and CDT corrected D-values.
[2] The values in parentheses are the regression of determination (R^2).
[3] Predicted values.

Since there has been no report on microwave destruction kinetics of these two strains, comparison of microwave kinetic data was difficult. However, the destruction behavior showed trends similar to that observed during conventional thermal destruction with D-values for *S. cerevisiae* being lower than that of *L. plantarum*. The magnitude of D-values, however, were considerably smaller for microwave destruction. The z-value of *L. plantarum* was smaller than that of *S. cerevisiae* (Figure 5.7) indicating the greater

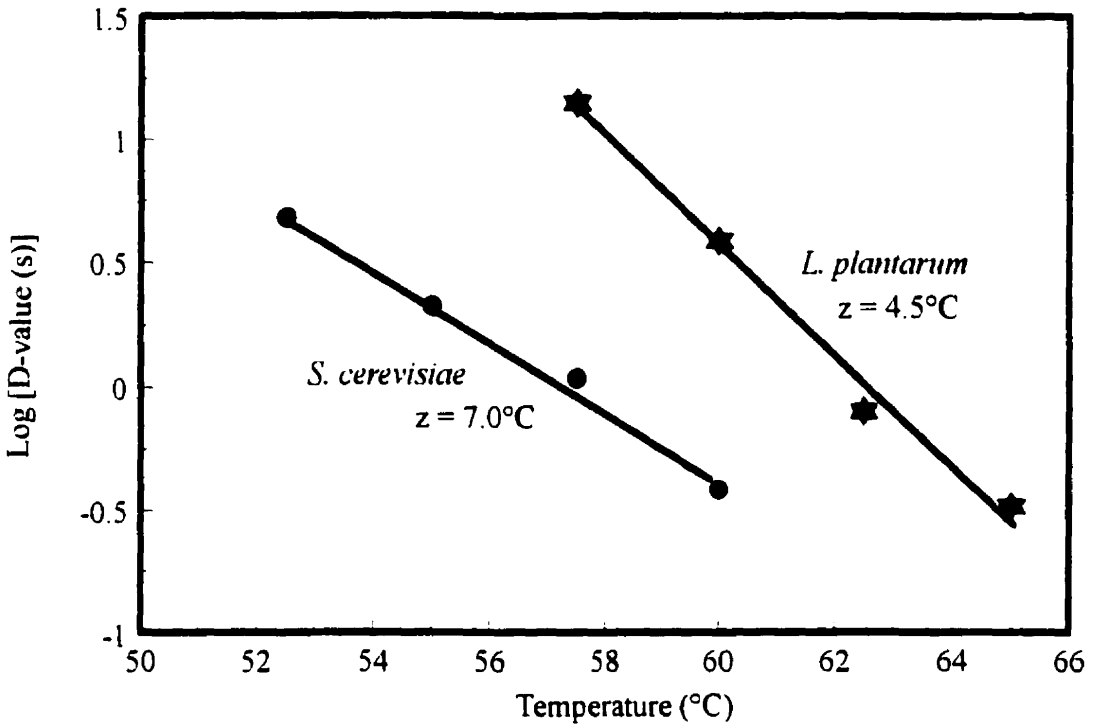

Figure 5.7 Temperature sensitivity curves of *S. cerevisiae* and *L. plantarum* in apple juice (pH 3.4) during continuous-flow microwave heating.

sensitivity of its D-values to temperature which could cause the reversal of magnitudinal differences of D-values between *S. cerevisiae* and *L. plantarum* at temperatures above 70°C. The reported z-values are closer to literature values (Kopelman and Schayer, 1976; Splittstoesser *et al.*, 1986; Török and King Jr., 1991; Garza *et al.*, 1994; Shomer *et al.*, 1994)

Comparison with thermal destruction

Comparison of microwave and conventional thermal treatments on the survival of *S. cerevisiae* and *L. plantarum* in apple juice at different temperatures indicated that the D-values obtained following microwave heating were considerably lower than those obtained following conventional thermal treatments. In Chapter III, it was reported that the D-values for *S. cerevisiae* ranged from 57.8 s at 50°C to 1.89 s at 70°C (Table 3.6). For microwave heating, the values ranged from 4.75 s at 52.5°C to 0.378 s at 60°C. At an intermediate temperature of 55°C, the D-values were 25.1 s for thermal destruction and 2.08 s for microwave destruction, the former about 12 times higher which indicated the microwave destruction rate to be about 12 times faster than the conventional thermal destruction. For *L. plantarum*, the thermal D-values ranged from 52.5 s at 55°C to 1.20 s at 80°C as compared to D-values in a range of 14.1 s at 57.5°C to 0.327 s at 65°C under microwave heating conditions. At the intermediate temperature of 60°C, the D-values were 21.9 s and 3.83 s, respectively, under thermal and microwave heating modes, again indicating the destruction rate under microwave heating mode to be almost 6 times faster. The effect appeared to increase with an increase in temperature for both microorganisms. This indicated that microwave heating was more efficient in destroying microorganisms in apple juice than thermal treatment as also observed with enzyme inactivation studies discussed in the previous section. The mechanism by which microwaves contribute to microbial destruction, other than the heat damage to cells, is still unclear. As noted earlier, the hypothesis of the additional microwave effects has been challenged by a number of researchers.

Sensory evaluation

As with the orange juice, the sensory evaluation was performed to test the differences in quality of conventionally pasteurized apple juice with and without the additional microwave treatment for an effective time of 3.6 s at 65°C. Eleven of the 30 panelists correctly identified the odd sample. According to the table for triangle test by Roessler *et al.* (1978), this indicated no statistically significant difference ($p > 0.05$) between the test samples. This confirmed that flavor profile of the juice was not adversely affected by the additional microwave treatment.

CONCLUSIONS

The application of microwave energy for inactivation of PME in orange juice and destruction of spoilage microorganisms (*S. cerevisiae* and *L. plantarum*) in apple juice were explored. The inactivation/destruction followed typical first-order reaction kinetics showing linear destruction rate on a logarithmic plot of residual activity/survivors vs. residence times. Residence times gathered during the microwave heating were corrected to accommodate the effective portion of CUT and contributory thermal inactivation/destruction during CDT. Microwave inactivation of PME was characterized by a reference D-value at 60°C of 12.4 s with a z-value of 10.2°C. Reference D-values at 55°C were 2.14 s with a z-value of 7.0°C for *S. cerevisiae* and 3.68 s at 60°C with a z-value of 4.5°C for *L. plantarum*. Within the range of temperatures and sample sizes employed in this study, microwave heating proved to inactivate the enzyme and destroy the microorganisms by an order of magnitude faster than conventional thermal heating. This suggests the existence of some contributory non-thermal effects with microwaves resulting in the higher rate of PME inactivation and microbial destruction as compared to conventional heating. Sensory evaluation showed that the microwave treatment resulted in no statistically significant differences in quality changes.

CHAPTER VI

MICROWAVE INACTIVATION KINETICS OF PECTIN METHYLESTERASE IN ORANGE JUICE UNDER BATCH-MODE HEATING CONDITIONS

ABSTRACT

Microwave inactivation kinetics of PME in single-strength orange juice were evaluated under batch-mode heating conditions. Different quantities of orange juice in a glass beaker (100 mL capacity) were heated in a conventional microwave oven for preselected time intervals to yield the desired final mass average temperatures (50, 55, 60 and 65°C). The effective portions of residence time during the heating and contributory inactivation during the cooling were accommodated for kinetic data handling. Based on residual PME activities and first-order rate of inactivation kinetics, kinetic parameters were evaluated. Results indicated that the microwave enzyme inactivation data were characterized by D-values of 61, 14, 8.9 and 3.4 s, respectively, at 50, 55, 60 and 65°C with a z-value of 12.7°C. Once again, PME inactivation in orange juice was significantly faster in the microwave heating mode than in conventional heating mode.

INTRODUCTION

Apparently, controversies exist with respect to contributory effects of microwaves. Several studies have reported enhanced microwave effects (Olsen *et al.*, 1966; Henderson *et al.*, 1975; Kermasha *et al.*, 1993ab), however, some reports do not support the occurrence of such effects (Goldblith and Wang, 1967; Goldblith *et al.*, 1968; Welt *et al.*, 1994). In the previous chapters, the thermal and microwave kinetics were evaluated for both enzyme inactivation and microbial destruction. Comparing the kinetics from the two different heating modes gave more than an order of magnitude difference in D-values for enzyme inactivation and microbial destruction. Microwave heating has, thus, shown better inactivation and destruction rates than the thermal heating mode. In the previous set-up

(Chapter V), the kinetic studies were carried out under continuous tube-flow microwave heating conditions. In order to confirm that this did not arise just from a system anomaly; the microwave heating was also carried out under batch mode since the conventional thermal inactivation studies were carried out under batch-mode heating. Microwave batch heating mode was thought to provide a more direct comparison between the two heating modes.

The objectives were, therefore, to (1) obtain kinetic data for PME inactivation in orange juice under batch-mode microwave heating conditions and (2) compare the results with those obtained from earlier studies.

MATERIALS AND METHODS

Microwave heat treatment

Test sample of orange juice (prepared as detailed in Chapter IV) were taken in a 100 mL cylindrical glass beaker (Pyrex® No.1000, 5 cm diameter). The beaker was centrally located inside microwave cavity and heated in a domestic microwave oven (Model RE-620TC, T. Eaton Co., Toronto, ON, 700 W, 2450 MHz) operated at full power. The beaker containing test samples were always positioned at an identical location at the center of the cavity. Starting with the same initial temperature of 10.0 ± 0.1°C, the sample sizes required to obtain selected heating times yielding several target temperatures (50, 55, 60 and 65°C) were obtained by trial and error. To obtain the final bulk temperature, the test sample was quickly mixed after heating and temperature was measured by using thin-wire (0.381 mm diameter) copper-constantan thermocouples (Omega Engineering Inc., Stamford, CT) and recorded using a continuous data acquisition unit (Dash-8, Metra-Byte Corp., Taunton, MA). The sample container was well insulated using Styrofoam™ to prevent heat loss during temperature measurement. Subsequent to heating, test samples were immediately cooled in an ice-water bath with constant mixing. Prior to running test samples for enzyme inactivation, several replicates were used to ensure a given volume of test sample heated for a given time interval gave nearly same final bulk temperature. In order to avoid interference with measurement of enzyme

activity, temperatures were not measured in test samples used for enzyme inactivation, but data were obtained from replicates under identical heating conditions.

Absorbed microwave power

The quantity of heat absorbed (Q) during microwave heating were evaluated using:

$$Q = [mC_p(T_f - T_i)]_{sample} + [mC_p(T_f - T_i)]_{beaker} + Heat\ Loss \qquad (6.1)$$

where: m = mass (kg); C_p = specific heat (kJ/kg°C); T_f = final bulk temperature (°C); T_i = initial temperature (°C). Heat loss was assumed to be negligible since the beaker was insulated. The calculated Q was converted to absorbed microwave power (P) by dividing by heating time. Subsequently, absorbed microwave power (P) was compared with the nominal microwave output (700 W). The density of orange juice was experimentally determined to be 1018 kg/m^3. Heat capacity of orange juice was calculated from Dickerson (1969) model as reported in Heldman and Singh (1981):

$$C_p = 1.675 + 0.025\ (\%\ Moisture) \qquad (6.2)$$

where % Moisture under the study was 90 %.

After microwave heat treatment, test samples were evaluated for residual PME activities as detailed in Chapter III.

Time-temperature profile determination

Time-temperature data of test samples during microwave heating were gathered using a Fluoroptic™ thermometer immersed in the juice and temperature readings were obtained via Luxtron (model 1000A, Mountain View, CA). The time-temperature data of the test samples were also gathered during cooling of preheated samples in an ice-water bath using the thermocouple-data logger set-up. The procedures employed for accommodating the effective portion of CUT during microwave heating, the CDT contributory inactivation during thermal cooling and kinetic handling of data under non-isothermal heating condition are detailed in Chapter V.

Come-up and come-down period correction

Figure 6.1 shows the time-temperature profile during the microwave heating and corresponding calculated lethality of orange juice during microwave heating. The time-temperature profile indicated essentially a linear come-up. Similar temperature profiles were observed during heating of orange juice of different volumes supporting observations by Prosetya and Datta (1991) and Datta *et al.* (1992). The CUT effectiveness was evaluated as the ratio of the effective time, t_e, from Eqn. (3.5) at the final bulk temperature (after correcting the D and z-values as detailed earlier) and total time assuming zero CUT (equal to the maximum residence time in this case). The CUT effectiveness values evaluated with reference to each final bulk temperature are summarized in Table 6.1 yielding relatively low values varying between 11 and 15 % depending upon the final temperature. As with the thermal inactivation kinetics (Chapter III), the effective heating times represent the effective portion of the total residence time and only the effective portions of the CUT as compiled Table 6.1 were taken for D-value calculations.

Figure 6.1 also shows a coupled cooling curve for the same condition. A comparison of the area under the heating and cooling curves for lethality gives an impression, at first glance, that cooling contributes more lethality than heating. What is not apparent in the figure is that under the microwave heating mode the CUT (~ 30 s) accounts for the entire duration of heating while the cooling time of ~ 30 s accounts for only a fraction of the total heating time normally required to cause enzyme inactivation. As mentioned earlier, any inactivation of PME during cooling was due to thermal contribution. Hence, although the come-down period was almost 50 % effective, its contribution to inactivation (based on kinetic data obtained earlier) was, in fact, very small. For example, at 60°C heating conditions (Figure 6.1), the effective microwave heating time (t_e) was 3.73 s, based on 12 % effectiveness for the CUT of 31 s (shown in the figure). This would give a D-value of 9.19 s (by a single-point determination approach). The effectiveness of cooling curve was almost 50 % of the 30 s CDT which

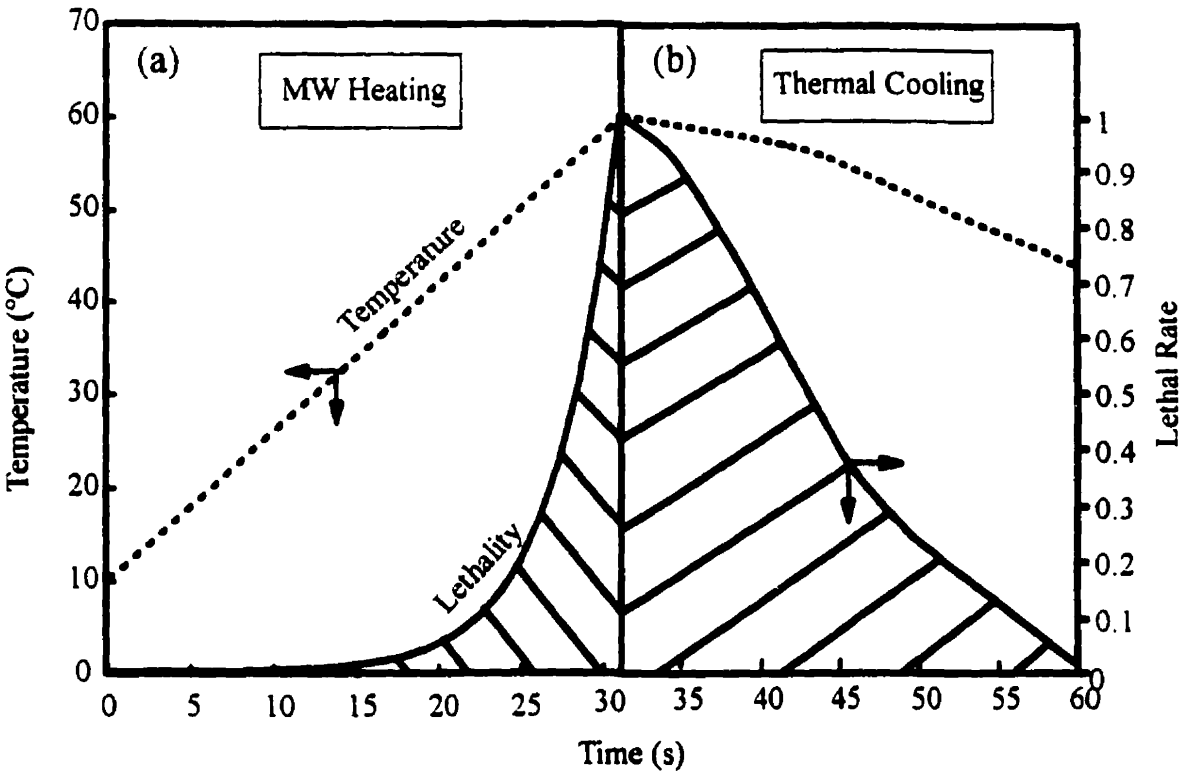

Figure 6.1 Typical time-temperature profiles of orange juice during (a) microwave heating and (b) thermal cooling and the corresponding lethal rates.

Table 6.1 Heating conditions for batch-mode microwave inactivation of PME in orange juice.

Final Temperature[1] (°C)	Sample Volume (mL)	Heating Time (s)	Effectiveness of CUT (%)	t_e (s)	t_c[2] (s)	Power Absorbed[3] (W)[4]	(W)[5]
50.2 ± 0.33	37.0	18	15.0	2.70	13.5	328	83.3
50.2 ± 0.29	56.5	26	15.0	3.90	14.6	347	57.7
50.5 ± 0.25	77.0	32	15.0	4.81	22.7	384	46.9
55.5 ± 0.29	30.0	18	13.4	2.40	9.91	300	93.7
55.2 ± 0.33	42.1	21	13.4	2.80	9.91	360	80.3
55.7 ± 0.11	55.4	28	13.4	3.74	15.6	356	60.3
55.0 ± 0.07	67.5	33	13.4	4.41	12.7	368	51.1
60.1 ± 0.33	29.2	20	12.0	2.40	9.57	321	93.7
59.7 ± 0.20	54.8	31	12.0	3.73	14.2	388	60.5
60.2 ± 0.15	68.5	36	12.0	4.33	13.8	418	52.1
64.6 ± 0.23	31.0	22	10.9	2.41	8.90	310	93.7
65.1 ± 0.27	37.8	24	10.9	2.62	8.90	346	85.9

[1] Mean and standard deviation (n = 4).
[2] Calculated based on thermal kinetics and used for corrections of residual PME activity.
[3] Assuming no heat loss to the surroundings.
[4] Absorbed by sample.
[5] Absorbed by beaker.

is 14.2 s. Based on a D-value of 155 s at 60°C for thermal inactivation, this would give an inactivation of about 5 %. The total inactivation observed for the condition was 60 % which would give a heating period inactivation of (60-5)% or 55 %, if cooling portion were to be excluded from the calculations. This results in a residual activity of 45 % from which the calculated D-value would be 10.6 s, slightly different from the previously calculated 9.19 s. While evaluating the microwave heating effects, the extent of inactivation which would have occurred during the cooling phase (as calculated by the effective thermal time during the cooling) was added to the residual activity so that the resulting activity would represent the residual activity following microwave heating only.

Microwave inactivation kinetics

Fresh orange juice containing naturally occurring PME subjected to microwave heating under conditions outlined in Table 6.1 was evaluated for residual activities following the microwave heat treatment. Figure 6.2 shows the activity and time-corrected PME inactivation curves at various temperatures under microwave heating conditions. The curves demonstrate that PME inactivation rate at higher temperatures is more rapid and, of equal importance, that PME inactivation kinetics under microwave heating complied with first order reaction. Evaluated D-values under microwave heating conditions are summarized in Table 6.2. The temperature sensitivity curve (log D vs. temperature) under microwave heating is included in Figure 6.3 and the calculated z-value was 12.7°C.

The microwave enzyme-inactivation kinetics under batch heating conditions evaluated in this study were limited only up to 65°C due to difficulties in obtaining consistent higher temperatures. Presuming no heat loss to surroundings, the microwave power absorbed accounted for 43 to 60 % as compared to nominal power (700 W) that can be given by microwaves. The small volumes of samples used in the study, therefore, did not absorb all the nominal power. Similar observation has been reported by other studies for small samples (Ramaswamy and Fakhouri, 1993). Within the range of experiments, the larger the sample volume, the higher the microwave power absorbed. The amount of absorbed power was, however, immaterial with respect to PME inactivation as the heating times were adjusted to give the same final temperature.

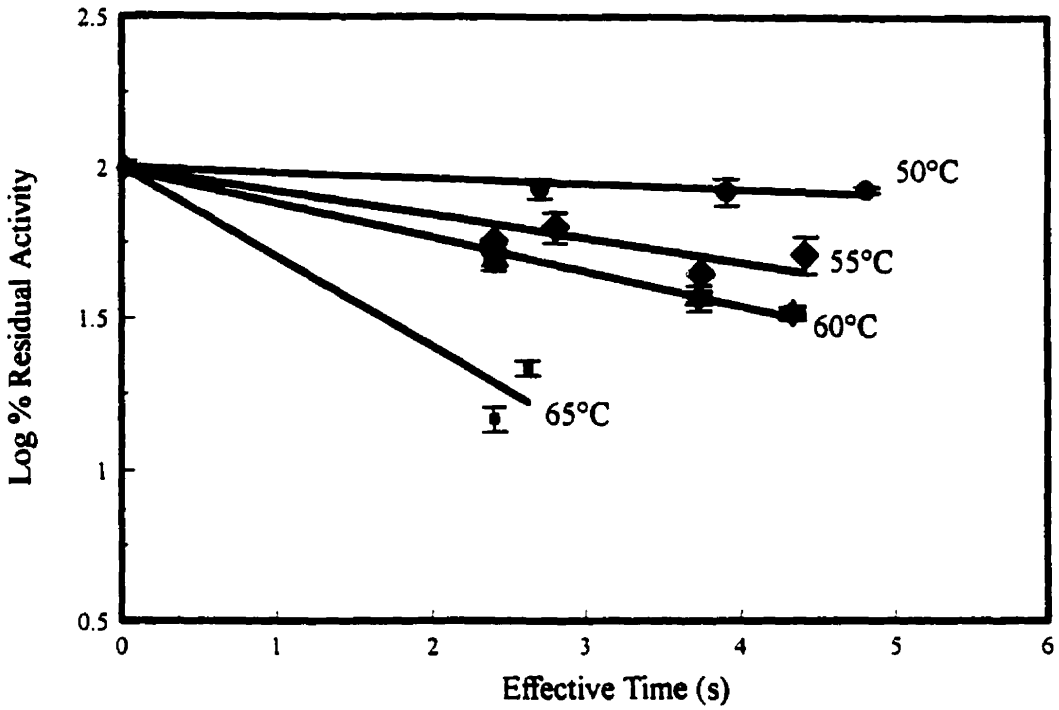

Figure 6.2 Semi-logarithmic plots of residual PME activity vs. effective heating time as a function of effective microwave heating times at various temperatures during batch-mode microwave heating.

Table 6.2. Microwave kinetic parameters (D and z) for PME in orange juice at various temperatures (pH 3.7 and 11.7 °Brix).

Temp. (°C)	D-value (s)					
	Uncorrected		CUT Corrected		CUT + CDT Corrected	
50	270	(0.93)[1]	40.5	(0.93)	61.1	(0.85)
55	87.7	(0.87)	11.7	(0.87)	13.6	(0.86)
60	61.3	(0.99)	7.37	(0.99)	8.91	(0.99)
65	27.0	(0.94)	2.96	(0.94)	3.44	(0.93)
z value (°C)	**15.8**	**(0.96)**	**13.4**	**(0.97)**	**12.7**	**(0.95)**

[1] The values in parentheses are the regression of determination (R^2).

Comparison with thermal inactivation

Comparing the D-values of PME inactivation under batch-mode microwave heating and conventional thermal heating modes, it was observed that the microwave heating was again remarkably more effective as indicated by considerably smaller D-values at any given temperature. The range of temperatures employed for thermal (60 to 90°C) and microwave (50 to 65°C) heating conditions were different due to (a) low inactivation rates at temperatures below 60°C during thermal mode and (b) difficulty in carrying out microwave heating experiments at temperatures beyond 65°C. At the common temperature of 60°C as the basis, the effectiveness of the two systems could be compared based on their D-values, i.e., 155 s during thermal and 8.91 s during microwave heat treatment. The two differed by more than an order of magnitude, in addition, this difference supported the previous observation with respect to the continuous-flow microwave heating showing the possibility of some contributory non-thermal effects of microwaves making it more effective than the conventional thermal treatment for PME inactivation.

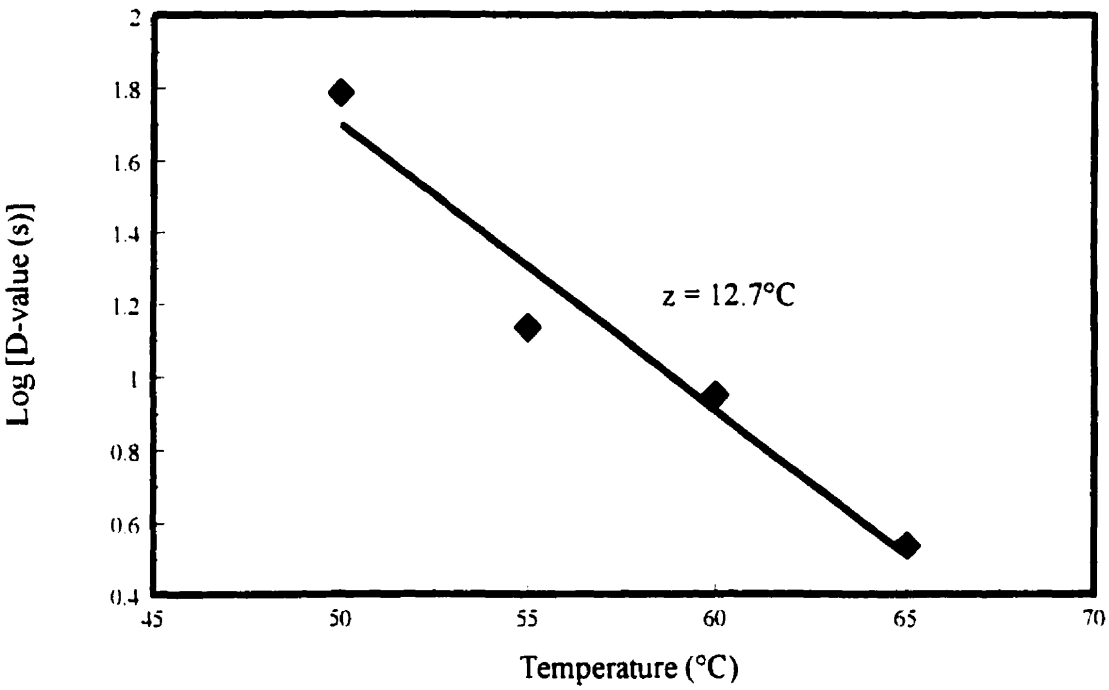

Figure 6.3 Temperature sensitivity curve of PME in orange juice (pH 3.7) during batch-mode microwave heating.

Source of error

A common source of error in this set of experiments is the repeatability of temperature conditions and nonuniformity of temperature within the test sample. Based on several replicate measurements, the variations in the bulk temperature following heating of test samples are shown as $\pm$ one standard deviations in Table 6.1. The variations were, therefore, no more than $\pm$ 1.0°C (taking $\pm$ 3 standard deviations) which is probably the case with even common water baths used in thermal inactivation studies. Error due to this margin of temperature difference will not account for the magnitude of difference observed between thermal and microwave heating modes (as discussed in the previous chapter) since the inactivation rates under microwave heating conditions at 60°C (D = 8.91 s) is five times faster than thermal inactivation rate even at 70°C (D = 37.5 s).

A second argument centers around the use of mass average temperature in these studies to represent the bulk temperature. The reported time-temperature and derived lethal rate profiles (Figure 6.1) obviously suggest non-linear dependencies. Thus, when enzyme inactivation is not linearly proportional to time and temperature, uneven heating and subsequent mixing would not necessarily give identical results to homogeneous heating. It is known that hot and cold spots or localized heating could occur during microwave heating of liquids especially with natural convective movement. It may be argued that the gathered time-temperature profile in this study may not represent the actual temperature exposure of the test sample at various locations in the beaker since it was recorded at a single point. The kinetic evaluation in this case was obtained based on the measured mass average temperature. In real situation, temperatures of the test samples in the beaker would be relatively non-uniform depending on the changes in dielectric properties with temperature, size of the sample, geometry of container, heating time and temperature achieved (Prosetya and Datta, 1991; Datta *et al.*, 1992; Anatheswaran and Liu, 1994). Knowledge of a range of enzyme inactivation changes with their associated volume fractions (a distribution) is the only way to provide complete kinetic analysis in this situation (which is not practical for experimental point of view).

Datta *et al.* (1992) have provided published simulated data on transient volumetric distributions of temperatures during unagitated batch microwave heating of liquids in a

cylindrical beaker. The test samples (500 mL) were filled into beakers of 11 cm in diameter to 10 cm in height. They reported some radial variation (2°C) and some axial variation (4°C) in temperature with temperatures increasing toward the top. Transient temperatures at any location were reported to be fairly linear and temperature differences were more apparent with heating time. The volumetric temperature distributions reported are shown in Figure 6.4.

It was not possible to measure the volumetric distributions of temperatures in this study. Therefore, data from Datta *et al.* (1992) (Figure 6.4) were used to evaluate the effect of non-uniform spacial and time-temperature distribution on the kinetic data gathered. The maximum temperature difference (taken from Datta *et al.* (1992) as $\pm$ 4°C) could be much shorter in the present set-up since the heating time, sample size and diameter of the container used were much smaller. Hence, this volumetric temperature distribution was considered fairly conservative. The following analysis was used to judge the appropriateness of the gathered kinetic parameters (D and z-values):

Step 1: A maximum spatial temperature difference of $\pm$ 4°C around the mass average temperature of 60°C was assumed based on Datta *et al.* (1992) and spatial distribution pattern for the temperature was set-up indicating the volume fraction of the sample associated (Row 1 and 2, Table 6.3). The distribution was adjusted slightly to give the target desired mass average temperature (60°C).

Step 2: Each volume fraction of the test sample was subjected to the heating time (CUT) at different temperatures (Row 3). The effectiveness of heating time (CUT) at different temperatures are different (Table 6.1). The effectiveness percentage of heating (Row 4) was used to compute the effective heating times (Row 5) at the various temperatures (56-64°C).

Step 3: Based on the kinetic data obtained from this study (D_{ref} (60°C) = 8.03 s, z = 12.7°C), D-values at different temperatures (56-64°C) was computed

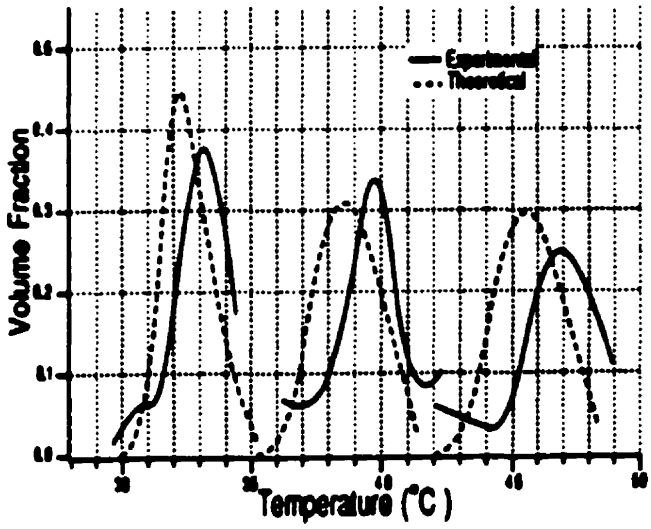

Figure 6.4 Volumetric distributions of temperatures of liquid during batch-mode microwave heating with natural convection (Source: Datta *et al.*, 1992).

Table 6.3 An analysis of the influence of spatial temperature distribution on kinetic parameters.

Temp. (°C)	56	57	58	59	60[1]	61	62	63	64
Volume Fraction[2]	0.01	0.05	0.10	0.18	0.32	0.18	0.10	0.05	0.01
Mass Average Temp.(°C)					60				
Heating Time (s)	20	20	20	20	20	20	20	20	20
Effectiveness (%)	13.1	12.8	12.5	12.3	12.0	11.8	11.6	11.4	11.1
Equivalent Time (s), t_e	2.61	2.56	2.51	2.46	2.41	2.36	2.31	2.27	2.23
D-value (s)[3]	16.6	13.8	11.5	9.63	**8.03**	6.70	5.59	4.66	3.88
Inactivation (%)[4]	30.4	34.7	39.3	44.4	**49.7**[5]	55.5	61.5	67.4	73.3
Mass Average Inactivation (%)[6]					49.8				
New D-value (s)[7]					**8.12**				
New Inactivation (%)					**49.7**				

[1] Average temperature, [2] Datta *et al.* (1992), [3] $D = D_{ref} \times 10^{(T_{ref}-T)/z}$; D_{ref} (60°C) = 8.03 s; z = 12.7°C. [4] % Inactivation = $100 - [10^{(2-(t_e/D))}]$, using t_e and D from the same temperature, [5] Extent of inactivation at mass average temperature of 60°C, [6] Average of (% Inactivation x volume fraction) from each temperature, [7] By trial and error.

(Row 6). Using the computed D-value and the equivalent heating time (Row 5), the extent of inactivation at each volume fraction was then computed (Row 7).

Step 4: From the distribution of extent of inactivation, the mass average inactivation (or the total inactivation) in the test sample was computed (Row 8), 49.8%.

Step 5: The extent of inactivation was then compared with the experimental value (49.7%). If the two matched, this meant that the D_{ref} and z from the study would adequately describe the inactivation kinetics under the test condition. If the two differed, the reference D-value (D_{60}) was either sequentially increased (8.12 s) or decreased until a match was obtained. The corrected value of 8.2 s, which woud give exactly the same amount of enzyme inactivation based on the assumed distribution, is only slightly different from the previously reported value.

This procedure was repeated for all experimental conditions. The resulting residual activities from the calculation were matched with those obtained experimentally for the same heating condition. A trial and error approach was used to estimate D-value that resulted in exact match of residual activity.

The range of D-values obtained varied from 50.0 to 50.3 s at 50°C (current value, 61.1 s), 20.2 to 21.1 s at 55°C (current value, 13.6 s), 7.98 to 8.16 s at 60°C (current value, 8.91 s) and 3.42 to 3.66 s at 65°C (current value, 3.44 s). Although the values reported in Table 6.2 may not represent the true kinetic values for PME inactivation during batch mode microwave heating, it is more than adequate to support the previous observation in Chapter V that the inactivation under microwave heating conditions are significantly superior to those obtained under conventional thermal heating conditions.

CONCLUSIONS

The possible challenge of employing microwaves for pasteurization of orange juice was further explored in this study using batch-mode microwave heating conditions. Sample of controlled sizes were carefully heated to selected times providing data for kinetic analysis at different temperatures. By applying the come-up and come-down period corrections, D-values of PME inactivation in single-strength orange juice obtained under batch-mode microwave heating were again remarkably smaller than those obtained from conventional thermal inactivation. This further indicates that microwave heating is more effective than conventional thermal heating in inactivating PME in orange juice. As discussed in the previous chapter, this can only be explained by some *non-thermal* microwave effects.

CHAPTER VII

EVALUATION OF ENHANCED THERMAL EFFECTS OF MICROWAVE HEATING ON ENZYME INACTIVATION AND MICROBIAL DESTRUCTION

ABSTRACT

This chapter is devoted to controlled evaluation of enhanced thermal effects under microwave heating. The inactivation of PME in orange juice and destruction of *S. cerevisiae* in apple juice were evaluated during microwave heating. Several techniques were employed for subjecting test samples to microwave heating: A) In the first set-up, test sample temperatures were maintained below 40°C while being subjected to full-power microwave heating conditions by surrounding the heating coil with a jacket through which cold kerosene (microwave-transparent liquid) was circulated. B) A second design permitted maintaining test samples at low temperature in a glass beaker; again while subjecting to full-power microwave heating conditions, by submerging a stainless steel helical heat exchanger in the sample through which cold water was circulated. Test sample in the beaker was kept well mixed through continuous circulation which permitted periodic withdrawal of samples. C) The third set-up was similar to B, but without the cooling coil; test samples were withdrawn after reaching selected temperatures.

Results from the first two set-ups showed that continuous microwave exposure of the orange juice and apple juice at low temperatures (< 40°C) did not have any major effect on inactivation of PME / destruction of *S. cerevisiae* even after heating for equivalent times which would have otherwise caused the entire sample to boil off. As the sample temperatures were increased in a step-wise manner (40 to 70°C) in the third set-up, the microwave heating gave consistently higher inactivation/destruction rates than conventional thermal heating. The degree of difference between microwave and conventional thermal heating (defined as the enhanced thermal effects of microwave heating) increased with temperature reaching more than an order of magnitude only at temperatures beyond 50°C. This enhanced thermal effect was proportional to the ratio of

mass equivalent microwave heating time and equivalent thermal time, which increased as the sample size decreased from 940 to 200 mL in the 700 W microwave oven. The microwave enhancement ratio (MER) which is defined as the ratio of the total to thermal inactivation/destruction on a logarithmic scale was observed to be temperature and sample size dependent.

INTRODUCTION

The applications of microwave heating in food processing have undergone intensively for more than 40 years (Decareau, 1985). The potential for microwaves to achieve pasteurization or even sterilization at lower temperature or in shorter time has been studied several times but the question remains if microwaves induce any changes in a manner that is not attributed to conventional heating.

In recent years, there have been several studies which suggest that microwaves have additional lethal effects other than those ascribed by heat. Olsen (1965) is probably among the first who postulated the existence of such effects. Some early work have attempted to determine the microwave effects without heating, by introducing dried samples to microwaves (Delany *et al.*, 1968; Vela and Wu, 1979). However, they reported no such apparent effects without the presence of water.

Some studies have been conducted to investigate the non-thermal microwave effects with pure microbial strains (Teixeira-Pinto *et al.*, 1960; Goldblith and Wang, 1967; Bluhm and Ordal, 1969; Fujikawa *et al.*, 1992). These studies have refuted the existence of non-thermal effects. Grecz *et al.* (1964) reported a greater destruction of *Clostridium sporogenes* spores heated in microwaves than that in equivalent conventional heating. Culkin and Fung (1975) reported a greater destruction of *Escherichia coli* and *Salmonella typhimurium* in microwaved-cooked soups and suggested that such a great deal of destruction over the conventional method can not be explained solely by thermal effects. However, Mertens and Knorr (1992) reported that the evidence was inconclusive since temperature non-uniformity most likely existed in the samples. Dreyfuss and Chipley (1980) studied such microwave effects on *Staphylococcus aureus*. The results indicated

changes in specific enzyme activity which was again not solely due to the thermal effects. Mertens and Knorr (1992) again reported that there was lack of thermal control samples for comparison. Khalil and Vilota (1988) compared cultures of *S. aureus* heated by microwaves and conventional method at 50°C and indicated a greater reduction by microwaves. However, Welt *et al.* (1994) showed no significant differences between microwave and thermal effects on the inactivation of the same spores. Their study was carried out in a microwave kinetics reactor in which the magnetron was adjusted to be on and off for controlling temperature. The microwave inactivation in this case would unquestionably be similar to the conventional thermal inactivation.

Some investigations have also been carried out with human serum enzymes (Beikhode *et al.*, 1974ab). More relevant studies have been carried out with several food enzymes like peroxidase, polyphenol oxidase, catalase, lipoxygenase, lipase and alpha-amylase (Lopez and Baganis, 1971; Henderson *et al.*, 1975; Esaka *et al.*, 1987; Kermasha *et al.*, 1993ab). The results from these studies were contradictory and inconclusive on the controversial issue of the existence of non-thermal microwave effects.

A technological advance in temperature measurement during microwave heating and a good control of temperature are necessary for this type of investigation. A temperature feed-back control system in a microwave oven was developed for controlling temperature within the oven based on the adjustment of on/off time of magnetron (Ramaswamy *et al.*, 1991; Tong *et al.*, 1993). However, this does not serve the purpose to demonstrate the non-thermal effects since any inactivation/destruction occurring during the microwave off-time would be considered the result of thermal injury. Another way of looking at this problem is to maintain low temperature at a level where thermal effects are negligible during the full microwave exposure by immediate removal of heat produced in the sample (Goldblith *et al.*, 1968; Lechowich *et al.*, 1969; Henderson *et al.*, 1975; Khalil and Vilota, 1988). This can easily be carried out using a material with low dielectric constant, e.g., kerosene or carbon tetrachloride, as an indirect coolant.

As can be seen from the preceding review, it appears that although several studies have been carried out to evaluate the existence of non-thermal effects under microwave heating, the results have not been conclusive. Even where there have been reported

evidence, the results have been generally refuted due to lack of accurate data on sample temperatures and/or temperature uniformity, inadequate equipment and techniques or accounting of temperature contributions between microwave and conventionally treated samples.

The objective of this study was specifically to evaluate the non-thermal or enhanced thermal effects of microwaves contributed to PME inactivation in orange juice and destruction of *S. cerevisiae* in apple juice using set-ups to maintain temperatures below 40°C under continuous and batch-mode heating (non-thermal effects) as well as under progressively increasing temperature conditions (enhanced thermal effects).

MATERIALS AND METHODS

Experimental set-up

A. Continuous-flow microwave treatment

The microwave oven (Model RE-620TC, T.Eaton Co., Toronto, ON) with 700 W and 2450 MHz was used throughout this study. In the first set-up, a specially-designed jacketed glass coil made of Pyrex® glass tubing was fabricated for accommodating heat transfer between the juice and a coolant within the oven cavity during continuous-flow microwave heating. The purpose was to maintain a lower temperature for the flowing juice during microwave heating by removal of heat produced in the juice using a microwave-transparent coolant flowing in the jacket surrounding the heating coil. The glass coil used had dimensions of 9.6 mm i.d., 1.545 m length. Similar to the continuous-flow microwave heating system detailed in Chapter V, the test sample was circulated through the inner helical glass coil (110 mL capacity) located centrally in the microwave cavity. The coolant was circulated in the jacket compartment of the glass coil and was externally cooled in a copper cooling coil outside the microwave oven prior to recirculating back to the jacket compartment of the coil using a Veristaltic metering pump (Monostat Corp., New York, NY). Kerosene was selected since it has been reported to be relatively microwave-transparent and absorbs only a small amount of microwave energy (Lechowich *et al.*, 1969). Inlet and outlet temperatures of both test sample and

kerosene were monitored continuously using thin-wire (0.381 mm diameter) copper-constantan thermocouples (Omega Engineering, Inc., Stamford, CT) positioned within the tubing just outside the microwave cavity. Temperature readings were obtained via a data-logger (Dash-8, Metra-Byte Corp., Taunton, MA). The flow rates of the juice (435 mL/min) and kerosene (3.2 L/min) were high to maintain juice temperature at the outlet port below 40°C at which thermal inactivation of PME was expected to be insignificant. A schematic diagram of the apparatus is shown in Figure 7.1.

B. Batch-mode microwave treatment with continuous-flow mixing

In the second set-up, microwave heating was carried out in a batch mode with a larger size of sample with continuous-flow maintained for mixing and sample removal. A Pyrex® glass beaker (1000 mL capacity) was modified to have an outlet glass connector (1.81 mm i.d.) on the side wall near the bottom for drawing the test sample for circulation. A microwave-transparent plastic tubing (Norprene®, 9.6 mm i.d., 12.8 mm o.d., 120 mm length) was used to allow the test liquid to flow in and out of the cavity. Tygon® tubing (7.94 mm i.d., 14.3 mm o.d., 0.78 m length) was used outside the microwave cavity. Test sample was circulated in and out of the beaker using a Veristaltic metering pump (Monostat Corp., New York, NY) at a fixed rate (1.9 L/min) for achieving sample mixing. Temperatures at the inlet and outlet port located outside the oven were continuously monitored as previously described. In order to maintain a low temperature (< 40°C) for the juice in the beaker during microwave heating, a cooling coil made of stainless steel tubing (8.0 mm i.d., 2.2 m length) was fully submerged in the juice inside the test beaker for rapid removal of heat produced in the juice. Ice-chilled water (0-2°C) supplied from an ice bank to a 50 L reservoir was circulated through the cooling coil at a constant flow rate (2.0 L/min) using a metering pump (Asea Co., Sweden). Figure 7.2 shows a schematic diagram of the system. To avoid arcing problems during microwave heating, the stainless steel coil was grounded to the cavity wall. Test samples were withdrawn following heating up to 3 h for orange juice and 2 h for apple juice.

Temperature uniformity in the beaker was evaluated by measuring sample temperatures during heating using fiberoptic probes inserted at various positions in the

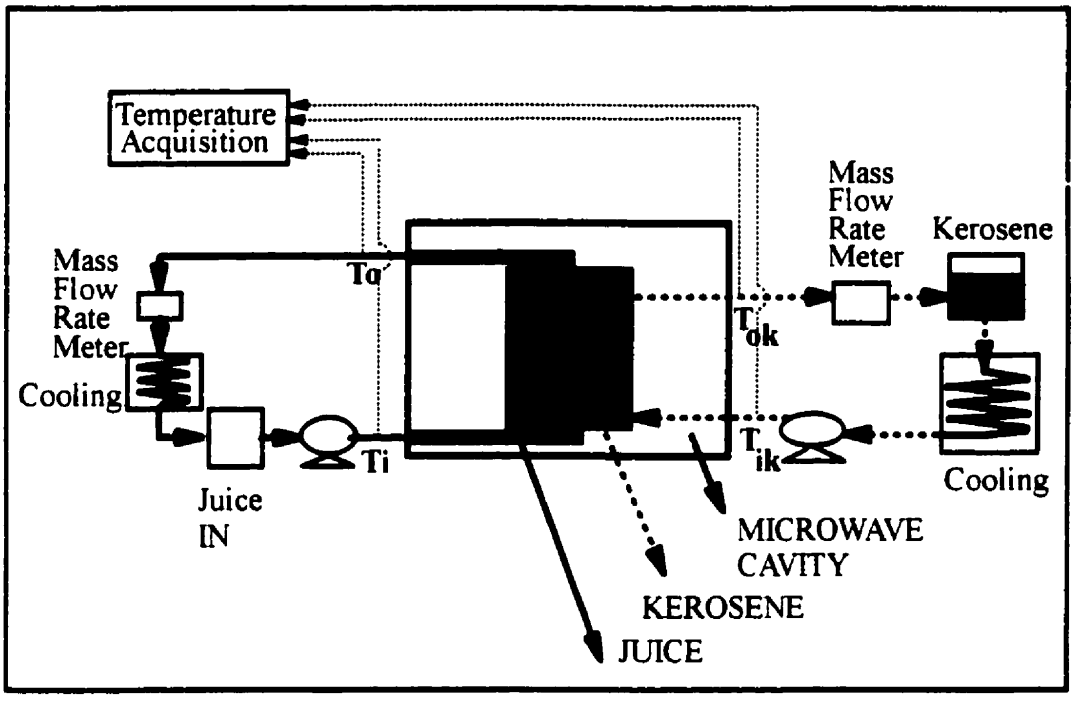

Figure 7.1 A schematic diagram of continuous-flow microwave heating system for evaluation of non-thermal effects at low temperatures.

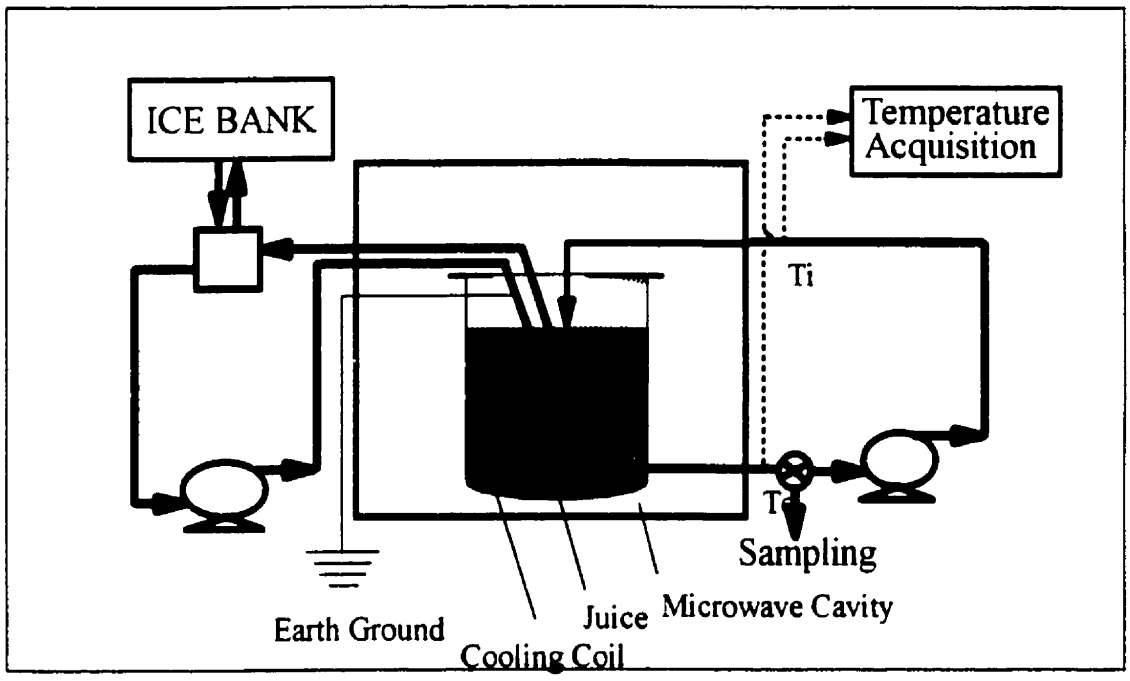

Figure 7.2 A schematic diagram of batch-mode microwave heating for evaluation of non-thermal effects at low temperatures.

beaker. Temperature readings were obtained by Luxtron (Model 1000A, Santa Clara, CA).

C. Batch-mode microwave treatment with progressively increasing temperatures

In the third set-up, the equipment used was similar to the set-up B, except for the removal of the cooling coil. Since there is no cooling, the sample temperature steadily increased during heating while the major portion of the circulating juice was continuously subjected to microwave heating. This permitted to maintain different levels of microwave and thermal exposure of the test samples. Time-temperature profiles of the juice for different volumes were continuously monitored. Test sample was withdrawn into a tube in an ice-water bath at the outlet port when the juice reached a selected temperature. For microbiological studies, heated apple juice samples with inoculated culture were drawn aseptically in an aseptic chamber as described earlier in Chapter V. Different test samples of 200, 400, 630, 800 and 940 mL were heated in the system and the samples were drawn at selected temperatures between 40 to 70°C.

Juice preparation

Orange and apple juice samples were prepared according to the details given in Chapter III. For apple juice, the calculated amount of the pre-culture (20 mL) was inoculated into 2 L apple juice to obtain an initial viable count of 10^6 CFU/mL.

Enzyme assay and enumeration of microorganisms

Residual PME activity in orange juice from the test samples was evaluated using a method adopted by Rouse and Atkins (1955) as described earlier. The liberated acids produced from a reaction of PME and pectin substrate were determined by titration using 0.02 mol/L NaOH as a titrant. A slightly modified method for using with an automatic titrator (Brinkmann Stat Titrino 718, Switzerland) was performed. Similar results were obtained using either automatic titrator or manual titration. PME unit was calculated from the slope of the straight portion of the titration curve (mL NaOH consumed/min) using Eqn.(3.1).

The number of *S. cerevisiae* survivors from both treated and untreated samples

were recovered on acidified PDA using spread-plate technique. Enumeration was performed as described earlier in Chapter III.

RESULTS AND DISCUSSION

Set-up A: Continuous-flow microwave treatment

Microwave heating was always carried out at full-power (100% power level, 700 W) for all operations in this study to give the maximum microwave input. Orange juice with enriched PME was used in the continuous-flow microwave heating system. Temperature stability in the system is illustrated in Figure 7.3 showing temperatures of the juice and kerosene as a function of microwave heating time. The low inlet temperature (~ 15°C) was maintained by circulating the juice through a glass cooling coil submerged in an ice-water bath in addition to the indirect heat exchange with kerosene. Temperature of kerosene was also maintained at ~ 10°C. Temperature of the juice at the microwave exit generally stabilized after a few minutes. It was necessary to separate lethal effects due to heat from those due to microwaves. Test sample was therefore continuously exposed to microwaves while the temperature was continuously maintained below 35°C. During the 90 min treatment time, 1200 mL of test sample was continuously circulated through the oven, but only a 110 mL portion was continuously exposed to microwaves. The total equivalent microwave exposure time for the 110 mL was therefore 8.25 min.

Percentage of PME inactivation in orange juice following exposure to microwave treatment at temperature where thermal inactivation was insignificant is demonstrated (Table 7.1). Inactivation during such exposure showed to be relatively insignificant (6.8%) as compared to that at higher temperatures. In terms of energy, the 90 min microwave heating at 700 W power would give (90 x 60 x 700/1000) = 3780 kJ of heat to the 1200 mL of orange juice. Assuming a heat capacity of 3.925 kJ/kg°C and a latent heat of 2200 kJ/kg, the 3780 kJ of heat absorbed would not only be sufficient to raise the temperature of the juice to boiling (100°C), but sufficient to completely boil it off (total energy required for all these is less than 3100 kJ). Hence, without the cooling heat exchanger, the microwave heating would have been more than enough not only to

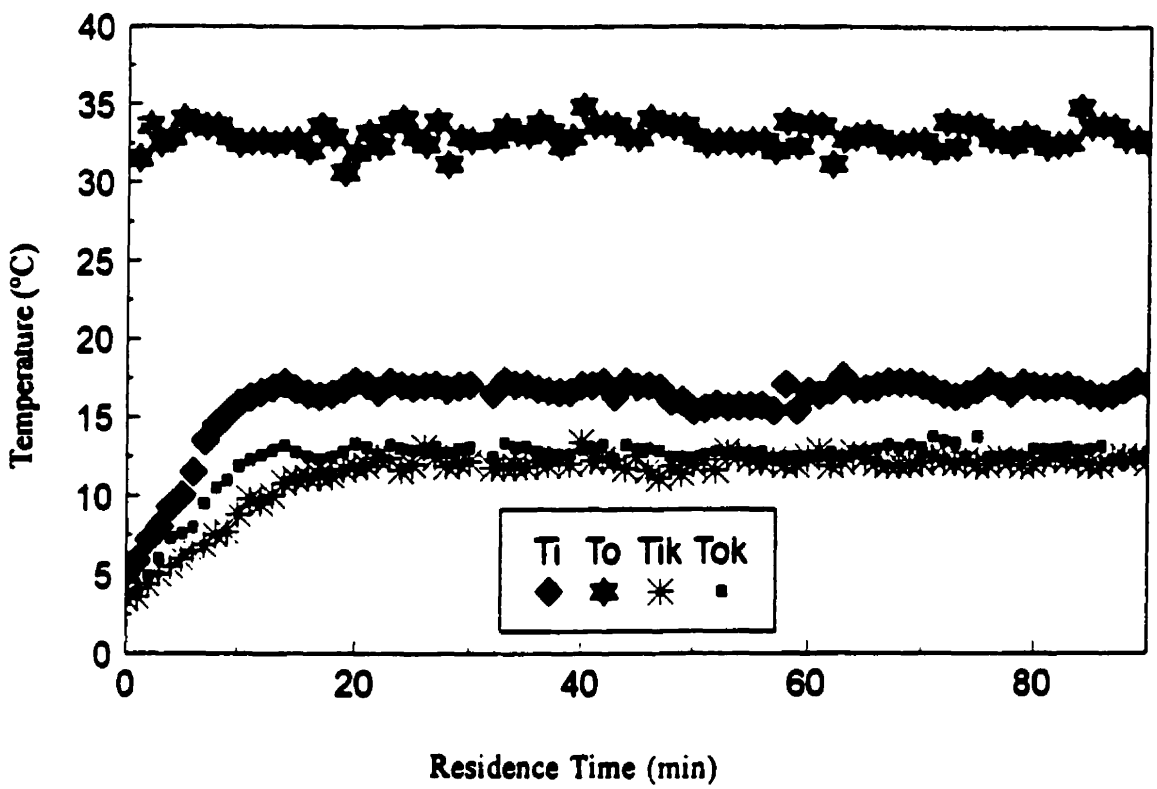

Figure 7.3 Typical time-temperature profiles of test samples (T_i and T_o) and kerosene (T_{ik} and T_{ok}) at inlet and outlet ports during continuous-flow microwave heating.

completely inactivate the enzyme, but also to evaporate the last drop of water in the orange juice. In view of these, the extent of inactivation observed were considered insignificant. Similar findings have been observed in some other studies when exposing samples to microwaves at sublethal temperatures (Goldblith *et al.*, 1968; Lechowich *et al.*, 1969; Welt and Tong, 1993).

Table 7.1 PME inactivation in orange juice following continuous-flow microwave heating (T < 35°C).

Time (min)	PME inactivation (%)
0	0
30	0.4
60	1.0
90	6.8

Set-up B: Batch-mode microwave treatment with continuous-flow mixing

Unlike in the microwave inactivation studies carried out under the batch-mode heating conditions (employing predetermined amounts of test samples and heating to a preselected time) described in Chapter VI, this system facilitated continuous mixing of samples. Since it was argued in the previous set-up (Chapter VI), temperature gradients as large as 4°C could exist in such system, it was considered prudent to install a mixing device. It was also necessary to draw the sample without interrupting the microwave heating conditions. Both were accomplished by the implemented recirculation system which kept the liquid in continuous circulation and facilitated sample withdrawal outside the cavity. Temperature uniformity of test sample in the beaker as measured using a fiberoptic probe indicated good stability (± 1°C).

The existence of non-thermal effects of microwave heating on enzyme inactivation were again evaluated at temperatures below 40°C to eliminate thermal effects. The

circulation of coolant in the stainless steel cooling coil located in the beaker allowed the juice temperature to be well kept below 40°C. Heat produced in the juice was rapidly removed. Tables 7.2 and 7.3 show PME inactivation rate in orange juice and destruction rate of *S. cerevisiae* in apple juice as a function of microwave exposure time, respectively.

Table 7.2 PME inactivation in orange juice following batch-mode microwave heating (800 mL, T < 40°C).

Time (h)	PME inactivation (%)	Absorbed energy (kJ/kg)
0	0	0
1.0	0.3	2658
1.5	0.3	4785
3.0	22	10366

Table 7.3 Survival number of *S. cerevisiae* in apple juice following batch-mode microwave heating (900 mL, T < 40°C).

Time (h)	Log reduction of survivors (CFU/mL)	Absorbed energy (kJ/kg)
0	0	0
2	0.2	5600

Results indicated that over a 3 h heating period, about 22 % inactivation of PME occurred, although it was only 0.3 % up to 1.5 h. The long time holding of juice at ~ 40°C could have been a contributing factor. The associated energy input as indicated in Table 7.2, was once again more than adequate to boil off the juice as detailed in the previous section. With respect to microbial destruction, the effect was even smaller showing less than one log cycle reduction in microbial survivors. Results, therefore, indicated that there were no appreciable lethal effects since inactivation/destruction appeared to be insignificant as compared with the inactivation/destruction that would have been obtained at higher temperatures. Lechowich *et al.* (1969) demonstrated similar findings with *S. cerevisiae* and *S. faecalis* suspensions at temperatures below 50°C. Welt and Tong (1993) observed no significant thiamine degradation upon microwave exposure (T<30°C) for 4 h. It was therefore concluded that there are no temperature-independent non-thermal effects associated with microwave heating when samples were held at temperatures below 40°C (set-up B).

Set-up C: Batch-mode microwave treatment with progressively increasing temperatures

In order to characterize the dramatic differences observed during the kinetic studies with respect to enzyme inactivation and microwave destruction between microwave and thermal heating modes, further investigations were performed using the same batch system employed earlier but without the cooling coil. Temperatures within the test beaker were again observed to be relatively uniform (± 1°C). Test samples were varied by size and temperature. In a non-isothermal heating condition, in this case, temperature of the samples linearly increased from ~20°C to 40, 50, 55, 60, 65 and 70°C. Samples (~2 mL) were only drawn when desired temperatures were reached. As expected the results showed that the heating rate was faster with samples of smaller size (Figure 7.4). The temperature difference achieved in the heated sample was plotted against mass normalized time (t_M) which is microwave heating time expressed per unit mass of sample (Figure 7.5). Theoretically, if the microwave energy were to be solely and fully used for heating the test samples and no heat losses occurred from test samples, the temperature difference achieved should be a linear function of the t_M with doubling of t_M resulting in a two-fold

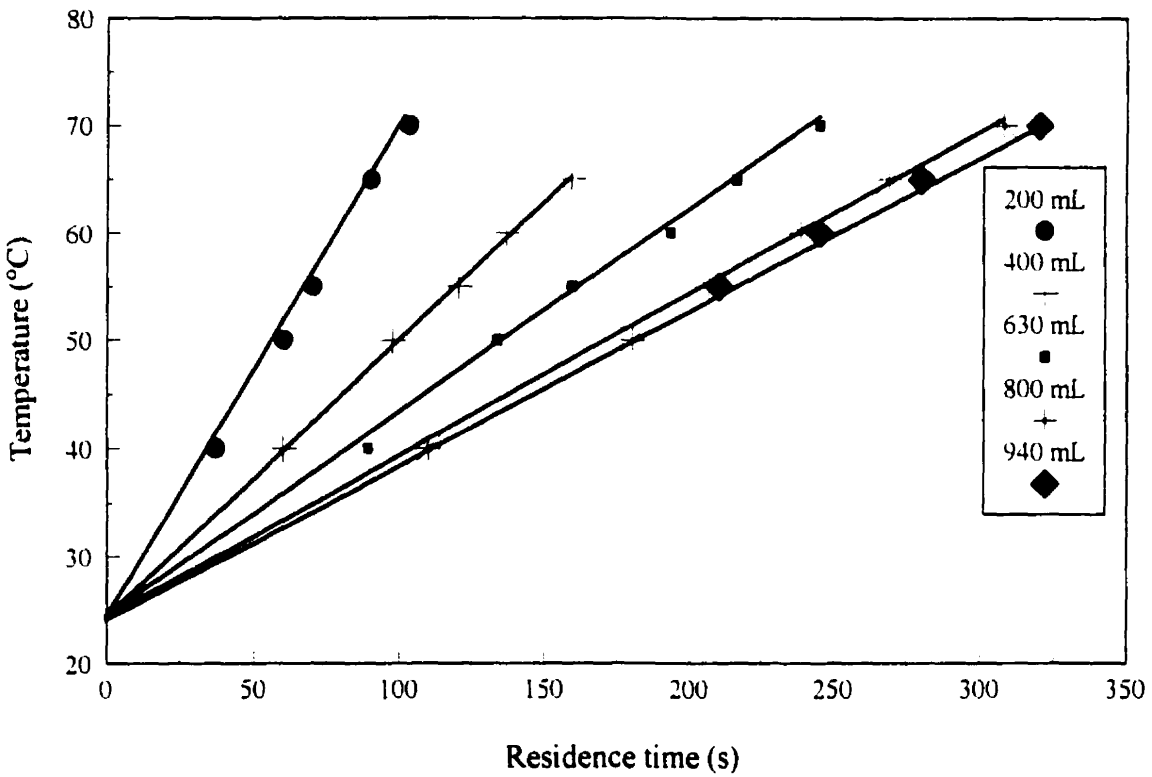

Figure 7.4 Typical time-temperature profiles of test samples in a batch mode microwave heating with progressively increasing temperatures at various sample sizes.

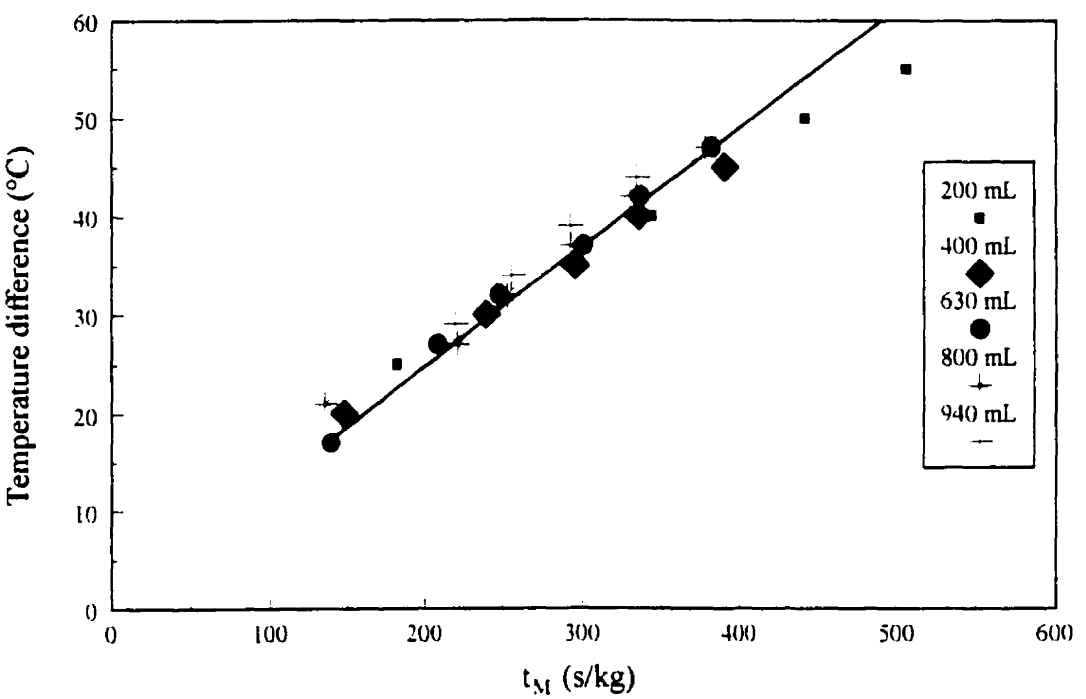

Figure 7.5 A plot of temperature rise as a function of mass normalized microwave time at sample sizes.

increase in the temperature difference. However, deviations from a linear relationship could be expected due to differences in energy absorption for different sample sizes and variations in the magnitude of heat losses. Results indicated some deviation with the smaller size of samples at higher temperatures. In a batch mode microwave heating, smaller sizes of samples (< 500 mL) have also been shown to absorb considerably less than the nominal power of the microwave oven (Ramaswamy and Fakhouri, 1993).

The inactivation of PME in orange juice and destruction *S. cerevisiae* in apple juice with progressive heating of test samples of various sizes are shown in Figure 7.6. Each datum point on a given curve (for a given sample size) represents a different target temperature ranging from 50 to 70°C. The time-temperature profiles up to each of these target temperatures were essentially linear as shown earlier. It should be noted that the inactivation/destruction shown is a combined effect resulting from both microwave and thermal contributions. In order to access the relative effects due to microwaves, the equivalent thermal effects for similar heat treatments needed to be calculated and deducted from the total contribution. Cooling corrections were excluded, since both thermal and microwave heating modes in this set-up would have same cooling correction. The thermal contribution was calculated based on the computed effective portion of the heating time using the previously determined thermal z-value (Chapter III). Once the effective thermal time (t_{eth}) is obtained, the extent of inactivation/destruction due to conventional "thermal" treatment could be found using the decimal reduction time at the temperature in equation: $10^{[2-(t_{eth})/D]}$. The equation represents the extent of inactivationédestruction at the thermal time, t_{eth}. The calculated amount was subtracted from the total inactivation/destruction to give the extent of inactivation/destruction caused by non-thermal microwave effect. The combined and individual effects of microwave and thermal treatments on PME inactivation and *S. cerevisiae* destruction are shown in Figure 7.7 and 7.8, respectively. Figure 7.7c and 7.8c, therefore, indicate the effects not explained by the equivalent thermal treatment. The magnitude of the difference were clearly found to be both temperature and sample size dependent. The difference between the two was more obvious with the smaller sample sizes. In addition, it was more obvious with PME at higher temperatures and with *S. cerevisiae* at lower temperatures. A careful examination

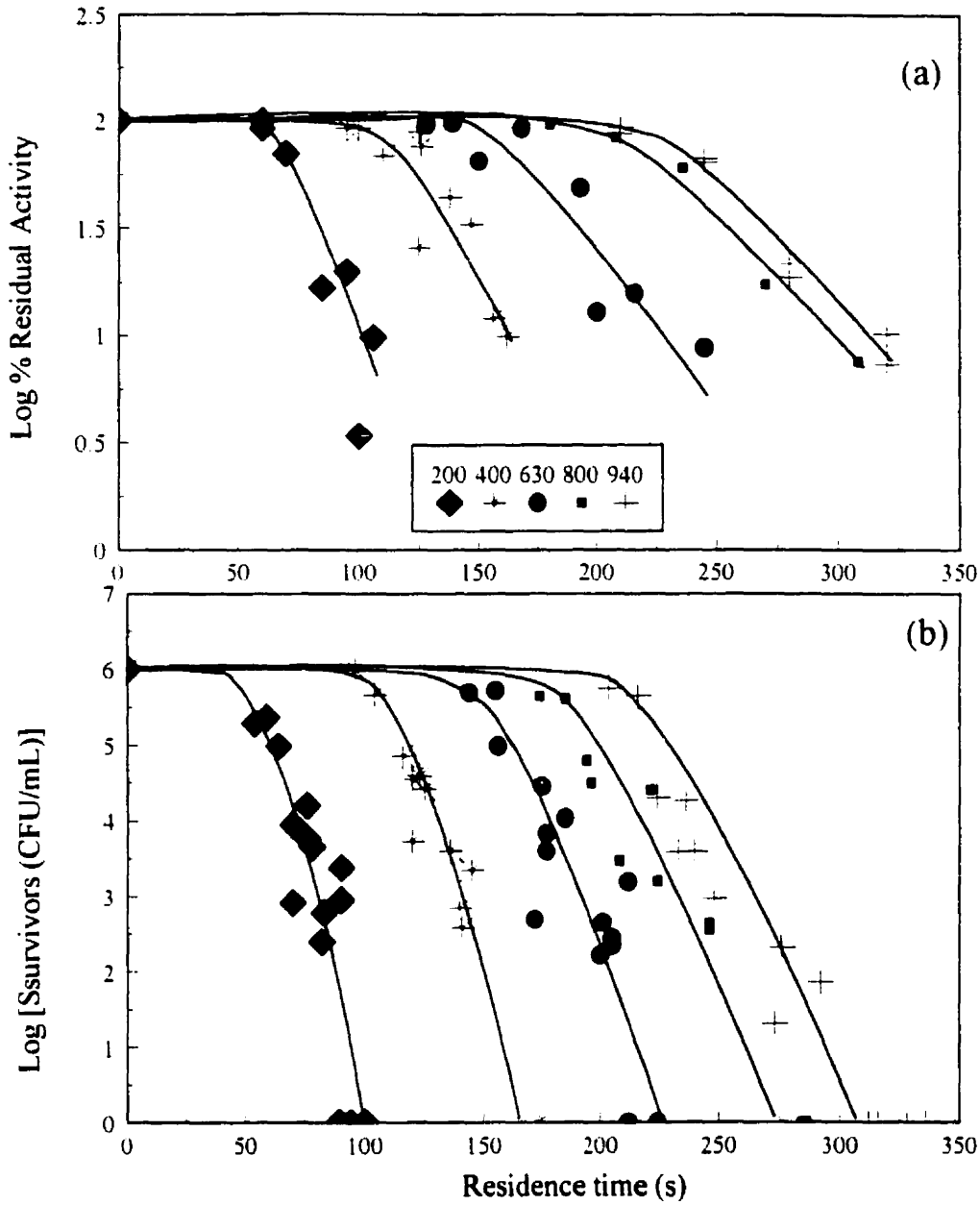

Figure 7.6 Microwave inactivation/destruction curves showing (a) inactivation rate of PME in orange juice and (b) destruction rate of *S. cerevisiae* in apple juice as a function of residence time at various temperatures and sample sizes.

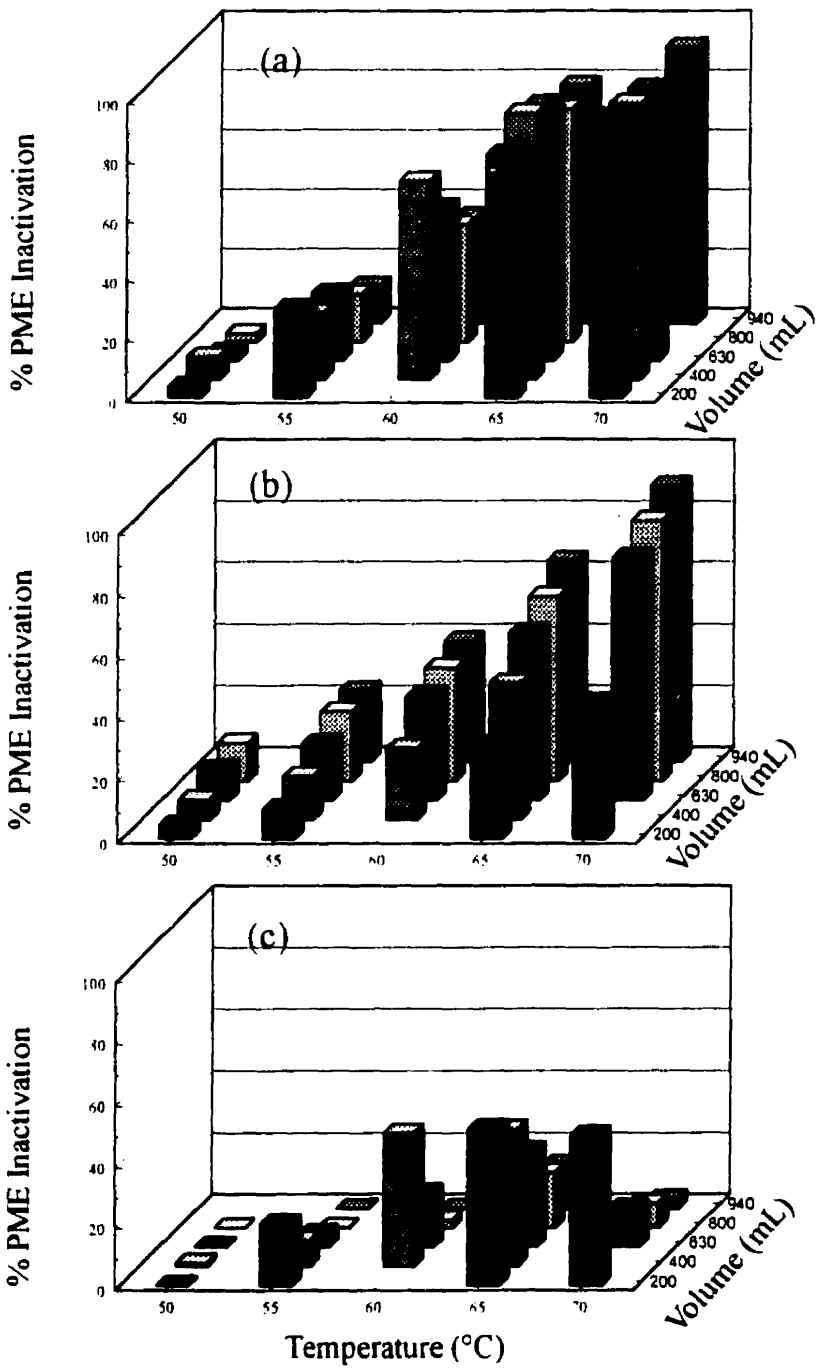

Figure 7.7 PME inactivation in orange juice as influenced by (a) microwave heating and (b) conventional thermal heating and (c) the differences of (a) and (b) at various sample sizes and temperatures [(c) represents additional microwave contribution].

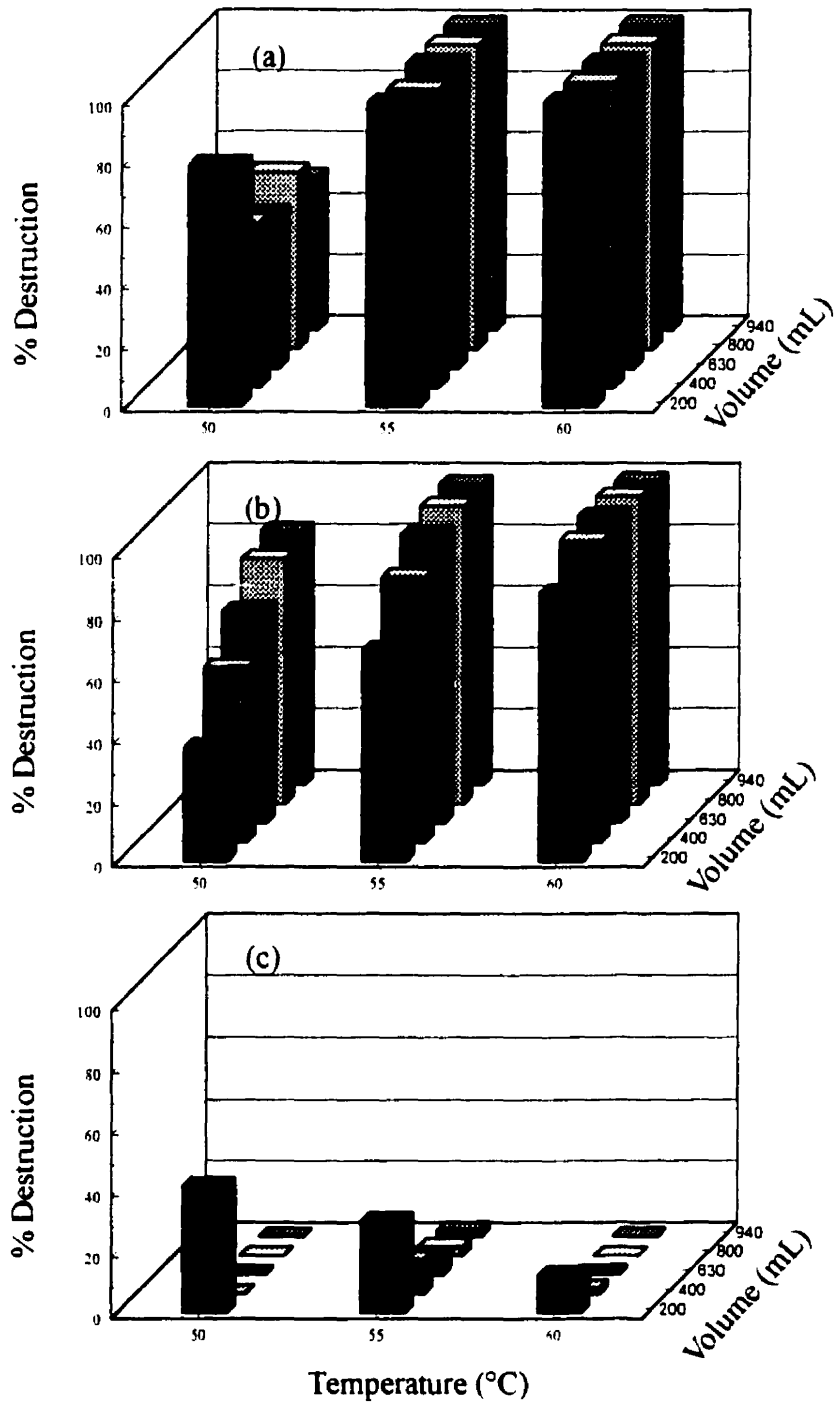

Figure 7.8 Destruction of *S. cerevisiae* in apple juice as influenced by (a) microwave heating, (b) conventional thermal heating and (c) the differences of (a) and (b) at various sample sizes and temperatures.

of the data indicated that the results are influenced not only by the factors influencing the inactivation/destruction but also by the availability of enzyme or microorganism for inactivation/destruction.

In order to more clearly understand the effects at different temperature and elucidate the differences between the thermal and microwave effects, several approaches were employed. The first step was to mass normalize the microwave treatment time which is a necessity with respect to microwave heating. This would give equivalent heating time for samples of different sizes. Since this step would cloud data points along the x-axis and since the objective was to compare the microwave effects with thermal effects, the relative microwave to thermal time (t_M/t_{eth}) was used as the basis. Theoretically, the t_M for a sample of two sizes is the same. The smaller sample would, however, have a shorter thermal time as compared with the larger sample because, with respect to thermal time, time is not normalized. Thus, smaller size of samples would have a relatively larger t_M/t_{eth}. It should be noted that both t_M and t_{eth} are the effective times, and not the real holding times, at the respective temperatures. The relationship between sample size and t_M/t_{eth} at various temperatures are shown in Figure 7.9. The second step was to adjust the y-scale such that the treatment would more clearly reflect the influence of the microwave treatment. Hence, the residual activity obtained after subtracting the thermal contribution from the initial activity was taken on the available initial activity for microwave only treatment. The percentage of inactivation/destruction due to the microwave only treatment was then computed as [(Total inactivation - Thermal inactivation) / (100 % - Thermal inactivation)] x 100. These are shown against t_M/t_{eth} in Figure 7.10 and 7.11, respectively for PME and *S. cerevisiae*. The results showed some common trend for the microwave effect. The extent of inactivation of enzyme increased with temperature (Figure 7.10), although some overlapping was observed between 65°C and 70°C. The reason for this overlapping was the apparent low levels of residual enzyme activity available for the microwave treatment after deducting the thermal contribution under these conditions. The available difference in activity was under 20% for all sample sizes except 200 mL. Since only this difference was used in computing "non-thermal" or the effects beyond what can be explained by thermal action, the results at these high temperatures were somewhat

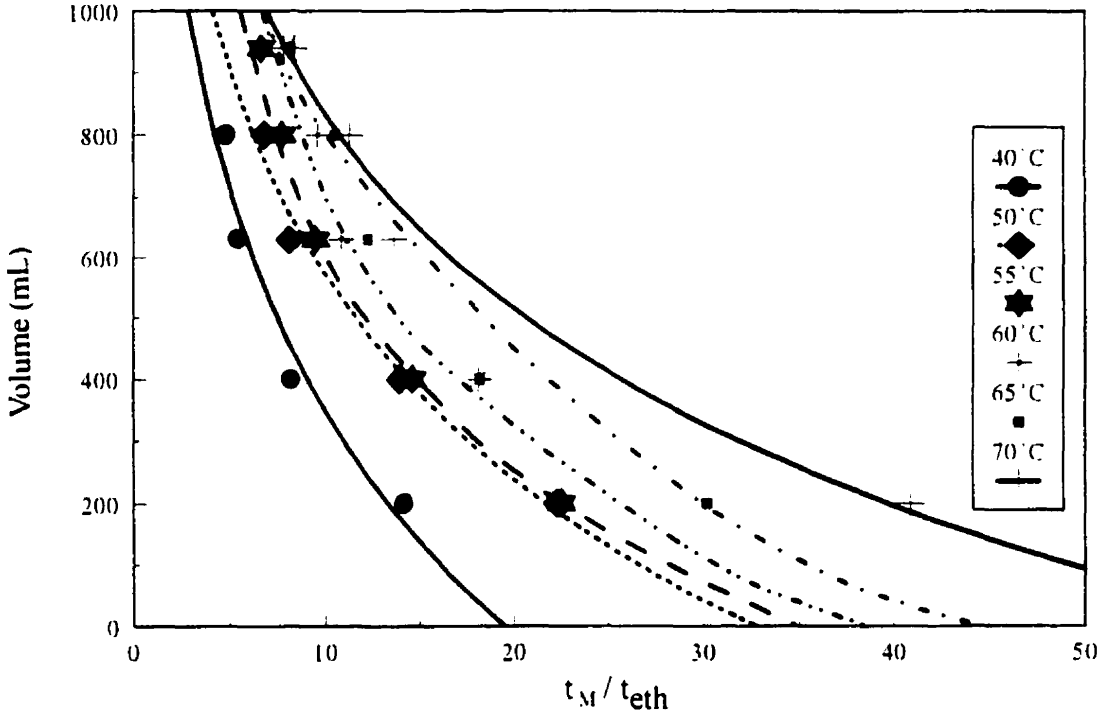

Figure 7.9 Ratio of mass normalized microwave heating time to effective thermal time (t_M/t_{eth}) as a function of sample size.

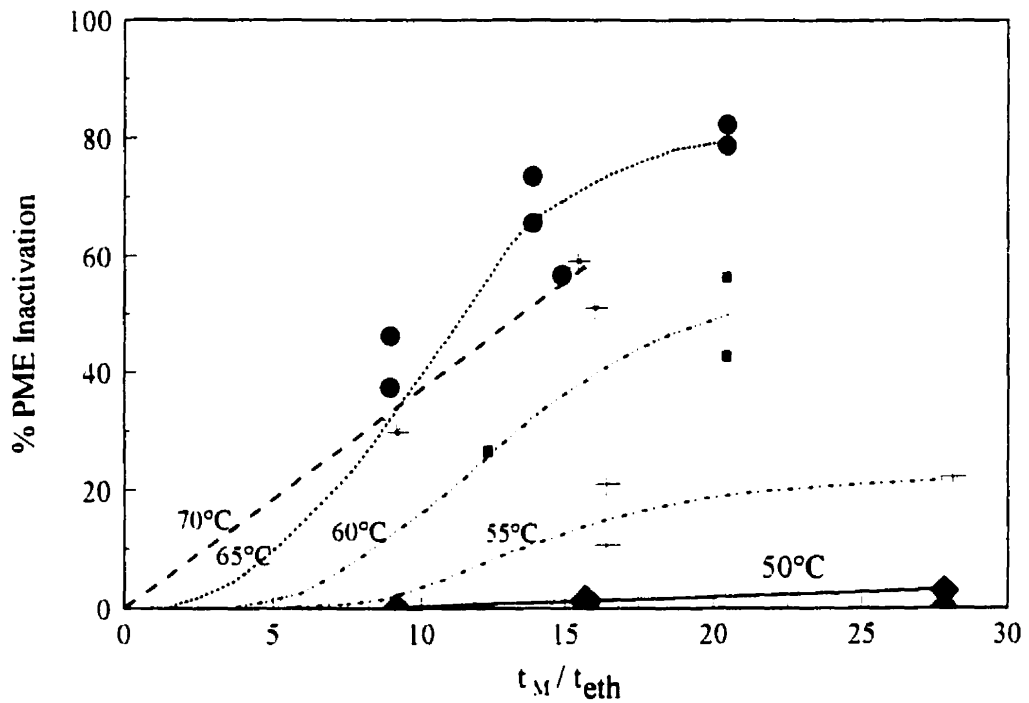

Figure 7.10 Enhanced thermal effects of microwave heating on PME inactivation in orange juice as a function of time at various temperatures.

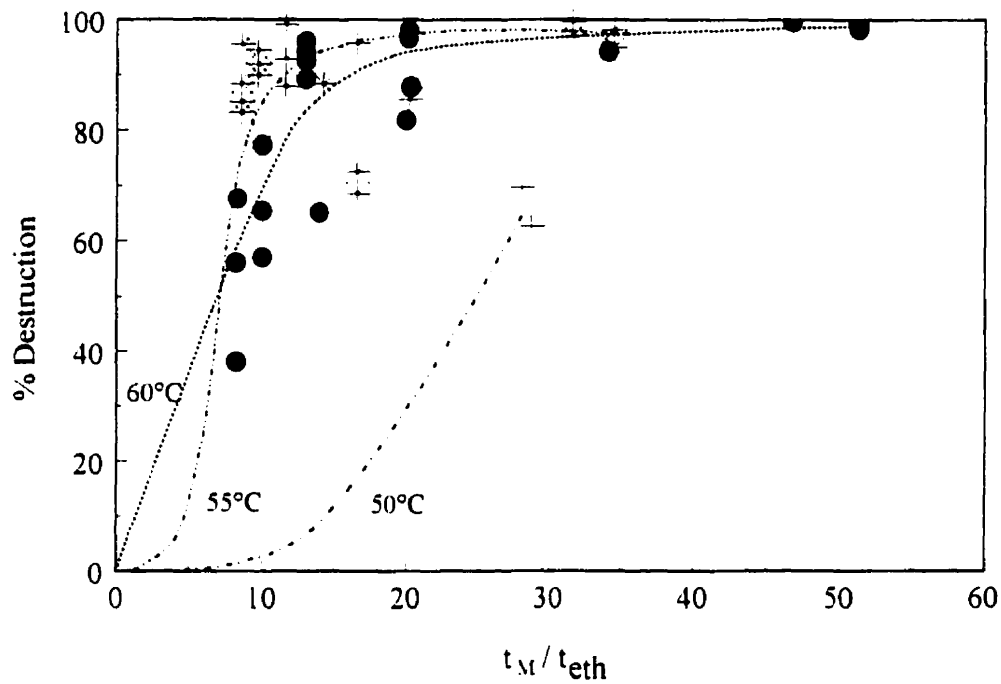

Figure 7.11 Enhanced thermal effects of microwave heating on destruction of *S. cerevisiae* in apple juice as a function of time at various temperature.

oversensitive and not properly accommodated. Other than these discrepancies, the results show similar trends of increased microwave effects at higher temperatures also with *S. cerevisiae* (Figure 7.11). This leads to the hypothesis that the observed microwave effects are not entirely "non-thermal", but are possibly temperature dependent. In the previous set-ups, it was shown that there were no microwave effects even after prolonged exposure at < 40°C. The results presented in Figures 7.6 to 7.11 also show that the effect was relatively very small up to 50°C. However, as the temperature increased beyond 50°C, the effects became more apparent and progressively increased with temperature. Hence, it was decided to use terminology "enhanced thermal effects" of microwaves rather than the traditionally used "non-thermal" effects. The data presented in Figures 7.7, 7.8, 7.10 and 7.11 also show that the effects are clearly sample size dependent. The t_{xt}/t_{eth} increases as the sample size gets smaller. Hence, the results were more obvious with smaller samples, which can easily be explained. The smaller size of samples received much shorter thermal time relative to the microwave exposure time. The larger size of samples received approximately the same microwave exposure time (on a mass normalized basis), but prolonged thermal time.

The preceding discussion points toward temperature related "enhanced" thermal effects rather than traditionally reported "non-thermal" effects. However, since the activities in Figures 7.10 and 7.11 were given in terms of % inactivation/destruction, they do not characterize the relative magnitude of enhanced inactivation/destruction effects. This relative magnitude was expressed in terms of a microwave enhancement ratio (MER) the ratio of total inactivation/destruction under microwave heating conditions (thermal plus microwave effects) to that calculated to be solely due to thermal treatment. The latter was previously calculated as $10^{(2-(t_e)/D)}$ using the equivalent thermal time (t_{eth}). To make a meaningful comparison for both enzyme inactivation (generally expressed as residual activity) and microbial destruction (as number of survivors), the ratio was computed using inactivation or destruction on a logarithmic scale. A value of the microwave enhancement ratio (MER) greater than 1.0 indicates the existence of enhanced thermal effects due to microwaves while the MER value of 1.0 or below shows its non-existence.

The enhanced microwave effects (MER value) of PME inactivation and destruction of *S. cerevisiae* are illustrated as a function of sample size and temperature in Figure 7.12. The effects were clearly more pronounced with smaller size of samples especially when addressing samples of small sizes (< 200 mL) employed in the preceding chapters. In addition, the effects were temperature dependent but generally not apparent at temperature below 50°C (with some exceptions). The destruction of *S. cerevisiae* appeared to be slightly less enhanced with the microwave heating as compared with enzyme inactivation. This may be due to the fact that *S. cerevisiae* is also quite sensitive to heat as was observed in Chapters III and V. In terms of the relative magnitude of MER for PME inactivation, the microwave heating was more than 20 times more effective than thermal inactivation at smaller sample sizes. With *S.cerevisiae*, the ratio was as high as 10.

The differential behavior of temperature-dependent microwave enhanced effects could arise from the fact that several properties that dictate temperature rise in the test samples under microwave heating conditions are temperature dependent, for example, dielectric properties, thermo-physical properties, etc. Furthermore, the heat produced in samples under microwave treatments results from the molecular friction which increases with temperature. These differences could make microwave heating considerably different from conventional heating and could very well cause some enhancement of the conventional temperature effects.

The inactivation/destruction resulting from the combined microwave and thermal contributions under various heating conditions as shown earlier in Figure 7.6 could be used to calculate the D-values as outlined in the preceding chapters. The iterative kinetic handling of data as detailed in previous chapters was employed for computing the effective heating times, t_e (which were a certain fraction of the total heating time). First, a z-value was computed by relating the total heating times to the residual activities, and the effective heating times were recalculated as the accumulated lethalities (Eqn. 3.5). These effective heating times were used to recalculate the D-values for each temperature and for each sample size. In this study, however, D-values were calculated based on a single time-residual activity or survival count data. For each sample size, several CUT corrected D-values were obtained at each temperature and the average values are shown

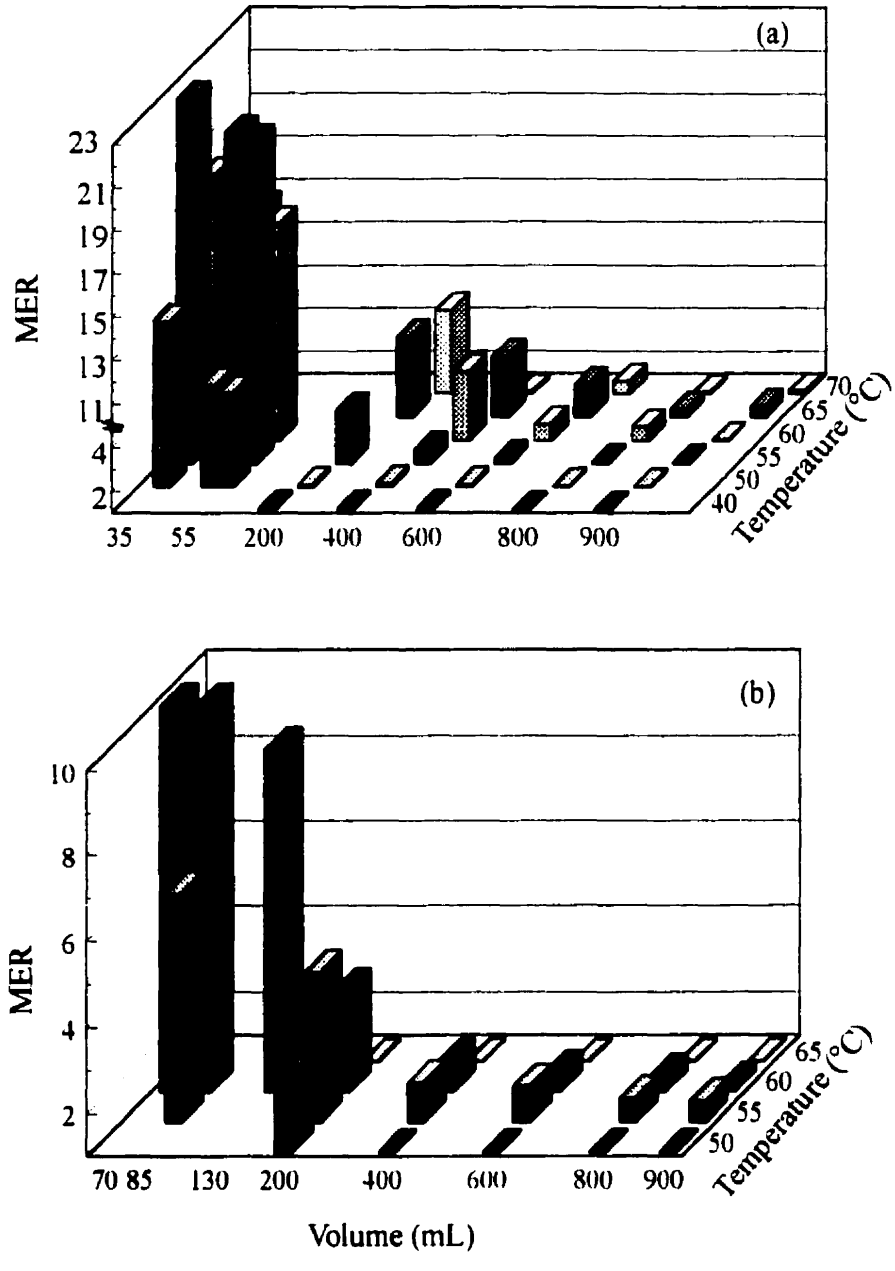

Figure 7.12 Microwave Enhancement Ratio (MER) for (a) PME inactivation in orange juice and (b) destruction of *S. cerevisiae* in apple juice as a function of sample size and temperatures.

in Figure 7.13 for PME and Figure 7.14 for *S. cerevisiae* which clearly indicate that, for both enzyme and microorganism, D-value was a function of sample size. It increased with an increase in sample size whereas the thermal effects were independent of sample size (shown as dotted lines). These were expected because microwave heating time is proportional to the sample size as well as microwave power level. Microwave heating time for any given set-point temperature or a given level of inactivation/destruction (such as D-value) will have to be longer for a sample of larger sample size. The figures also demonstrate a degree of difference between microwave heating mode and conventional heating mode up to a certain sample size at a given microwave power level. The D-values at different temperatures were 10 to 15 times higher for thermal heating than for microwave heating when sample sizes were below 100 mL and about 1 to 5 times at 500 mL. In addition, it was only marginally higher as sample sizes increased to about 1000 mL.

The absorption of microwave power is sample size dependent. As pointed in the previous chapter, smaller sizes of less than 500 g may not absorb the full-power supplied by the microwave oven. Based on temperature rise in the sample and associated temperature changes in the support system (glass beaker, plastic tubings, etc.), the calculated utilization of the nominal power of the oven is shown as a function of sample size in Figure 7.15a. The dotted line shows absorption efficiency of the test samples prior to accommodating the heat loss due to surroundings (beaker, tubing). As illustrated by the solid line, the absorption efficiency ranges from ~ 65% with smaller sizes to the maximum (100%) beyond sample size of 500 g in the 700 W microwave oven. Figure 7.15b gives a normalized version of microwave power absorption efficiency as a function of microwave nominal power (kW) for heating a unit size sample (1 kg).

Figure 7.16 gives the calculated D-values as a function of temperature for various sizes of samples, with those under conventional thermal treatment superimposed for both enzyme inactivation (Figure 7.16a) and microbial destruction (Figure 7.16b). The figures clearly indicate a region in which microwave heating offers advantage over conventional heating. It also demonstrates regions where microwave heating offers no benefit. Clearly, an understanding of what is happening is important in this situation. Without

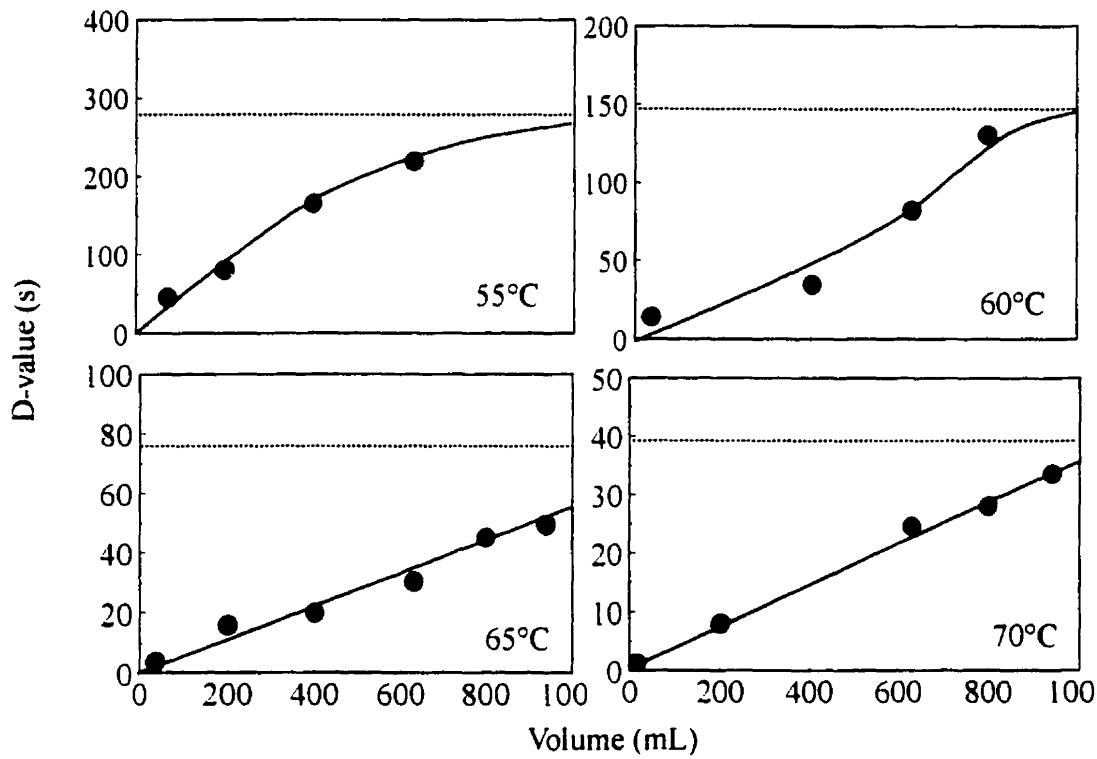

Figure 7.13 Decimal reduction times of PME in orange juice (pH 3.7) as influenced by volumes during microwave heating conditions at various temperatures (dotted lines show D-values for conventional thermal heating at respective temperature).

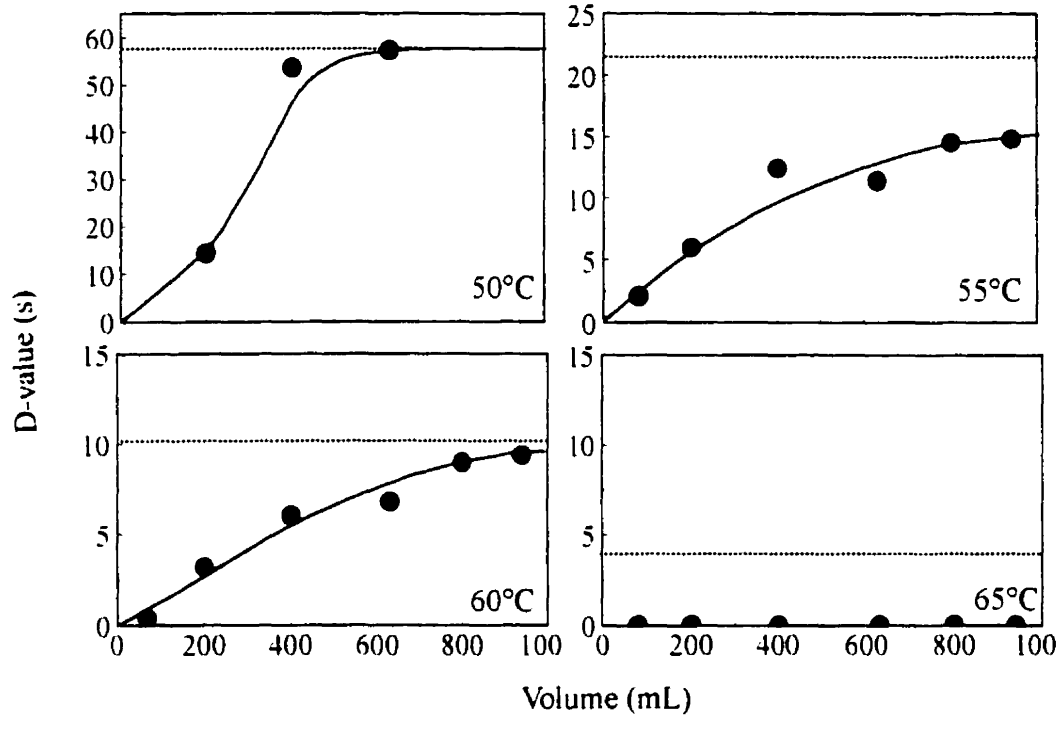

Figure 7.14 Decimal reduction times of *S. cerevisiae* in apple juice (pH 3.4) as influenced by volumes during batch mode microwave heating conditions at various temperatures (dotted lines show D-values for conventional thermal heating at respective temperature).

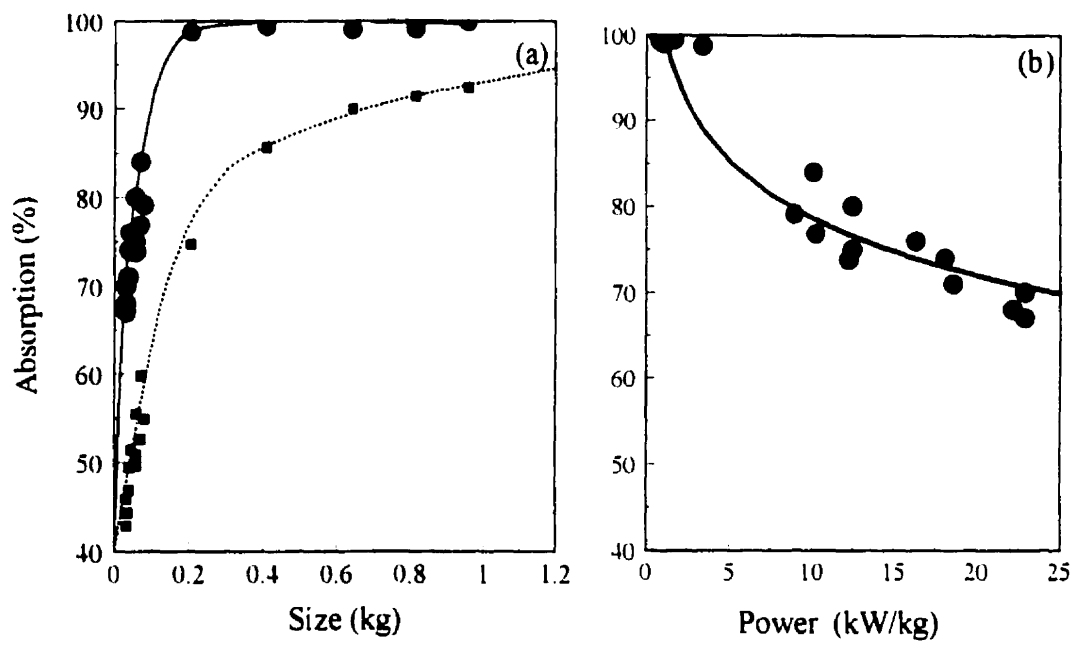

Figure 7.15 Percentage of microwave energy absorption as a function of (a) size at 700 W microwave power and (b) power for unit mass (kW/kg).

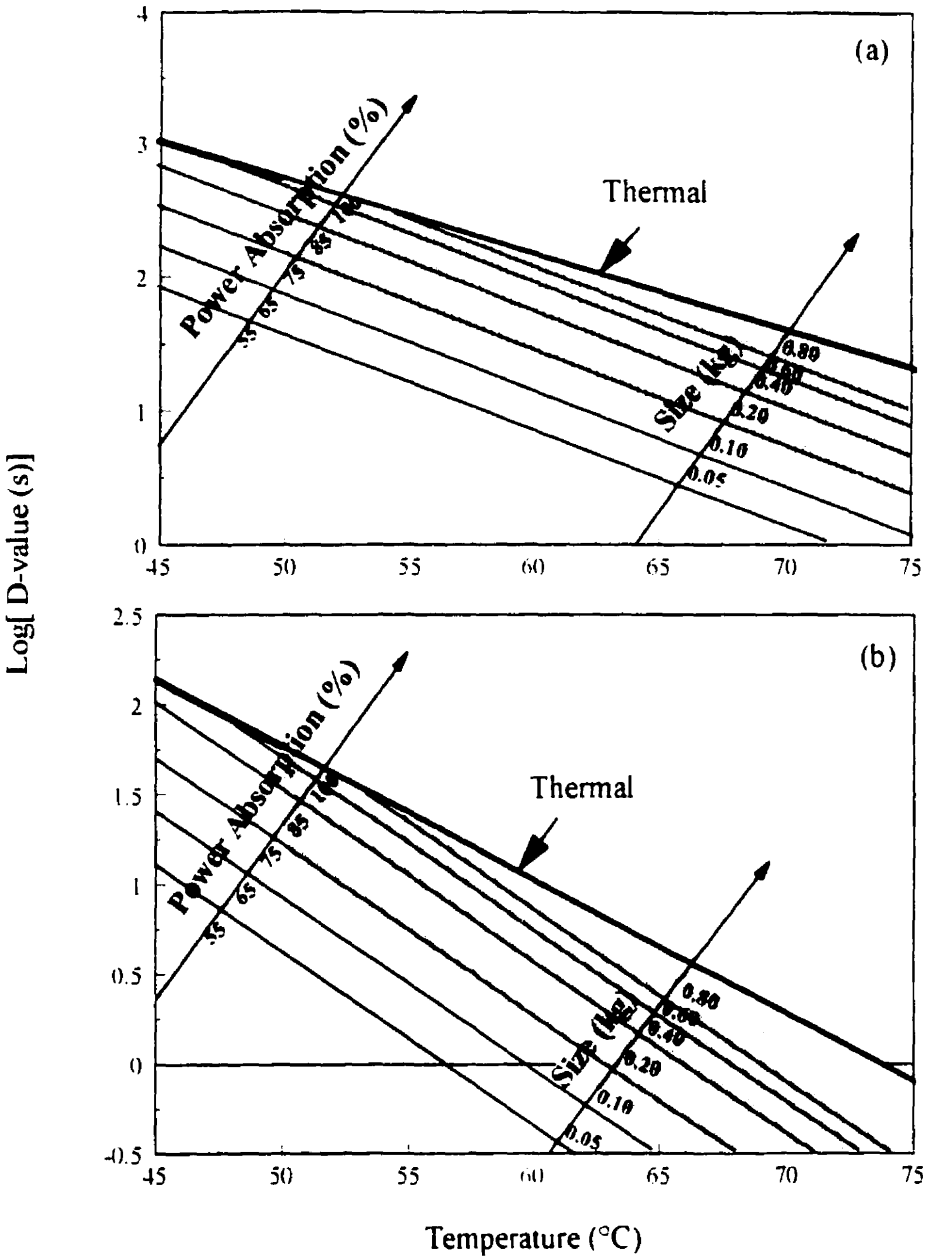

Figure 7.16 Figure depicting the optimal condition for positive enhancement effects of microwave heating in a 700 W microwave oven (a) for PME inactivation in orange juice and (b) destruction of *S. cerevisiae* in apple juice.

understanding the dependence of microwave heating time (and hence any D-values obtained) on the sample size, one could get the enhanced effect to be present or not present depending upon the sample size. Discrepancies observed in literature could have arisen due to non-recognition of these issues. Figure 7.16a indicates that the microwave heating would be beneficial for enzyme inactivation at sample sizes below 1000 g for a microwave oven of 700 W nominal power level in the temperature range of 55 to 70°C. Similarly for microbial destruction, the limit is below 1000 g in temperature range of 50 to 60°C. In figures 7.16a and 7.16b, also superimposed are the percentage absorption of the nominal microwave power. Clearly, at sample size below 150 g, the efficiency drops to below 70% level making it less attractive from economic standpoint. Using this value as the limiting condition, the zone for microwave advantage is indicated by the shaded region. In order to generalize the above concept, simulated conditions are provided in Figure 7.17 for both enzyme inactivation and microbial destruction representing D-values at various temperatures as a function of relative power, i. e., power in kW required per kg sample size (kW/kg). Again, the power absorption efficiency and thermal kinetics data are superimposed to highlight the area which provides enhancement of thermal effects under microwave heating conditions.

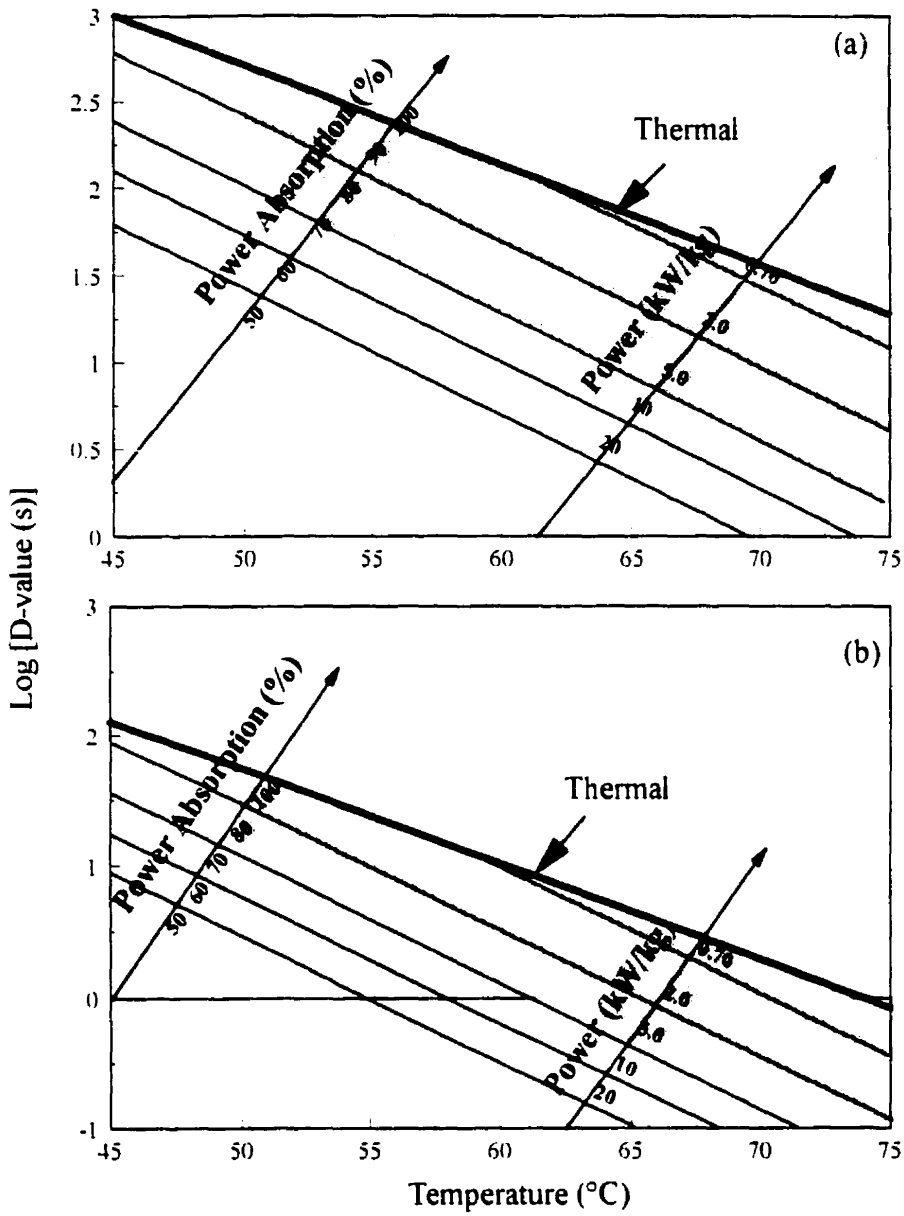

Figure 7.17 Figure depicting the optimal condition for positive enhancement effects of microwave heating at various microwave power capacities (kW/kg) (a) for PME inactivation in orange juice and (b) destruction of *S. cerevisiae* in apple juice.

CONCLUSIONS

Enhanced thermal effects of microwave heating on enzyme inactivation and microbial destruction in fruit juices were evaluated. Results obtained from the previous chapters suggested that microwave heating was an order of magnitude more effective than conventional thermal mode of heating. This implied some additional *non-thermal* effects associated with microwave heating giving greater enzyme inactivation and microbial destruction when compared with the conventional mode of heating. The existence of *non-thermal* effects with respect to microwave heating have been revealed in several studies but also have been refuted in several others. Results obtained in this study at low temperatures with continuous and batch-mode microwave heating at 2450 MHz and 700 W, indicates that the microwaves apparently produce no lethal effects on PME inactivation or microbial destruction. These results confirm several published studies conducted at sublethal temperatures on the non-existence of *non-thermal* microwave effects.

Since several properties of the test samples, e.g., dielectric properties, thermophysical properties, are temperature dependent, it was hypothesized that the differential phenomena existing in the two systems could be temperature dependent as well. Results of the study confirmed once again that there are additional effects resulting in enhancement of enzyme inactivation/microbial destruction due to microwave heating and this effect was dependent upon both sample size and temperature. These were characterized by employing the concepts of mass normalized microwave heating time to thermal time ratio (t_M/t_{eth}) and microwave enhancement ratio (MER) which is the ratio of microwave to thermal inactivation/destruction. The MER values were as high as 10 to 20 times for smaller sample sizes (< 100 g) and again were apparent only at temperatures beyond 50°C. The regions where microwave heating is more efficient than conventional thermal heating were identified for various sample sizes, temperatures and microwave capacities for both enzyme inactivation and microbial destruction.

CHAPTER VIII

CONCLUSIONS AND RECOMMENDATIONS

GENERAL CONCLUSIONS

This study has provided a basis for microwave pasteurization of fruit juices. Since very little information is available in the literature, the inactivation/destruction kinetics of enzyme and microorganisms which are required for establishing microwave pasteurization schedule were studied. A microwave heating system was developed and evaluated to accommodate a continuous flow of liquid foods and to study microwave inactivation/destruction kinetics for pasteurization purposes. The methodology for accommodating effective portions of the lag periods during heating and cooling was standardized in order to obtain more accurate kinetic data. Correction to heating and cooling times during come-up and come-down periods (lag) were applied by integration of cumulative lethality based on time-temperature history of the sample. It was necessary to appropriately compare the kinetics under the two heating modes with the similarly prepared juices. The thermal kinetics were, therefore, evaluated for the most heat resistant enzyme, PME, in orange juice and the most common spoilage yeast, *S. cerevisiae*, and spoilage bacteria, *L. plantarum*, in apple juice and used for comparison with the kinetics obtained during microwave heating.

The results on kinetic data indicated that microwave heating is superior to conventional thermal heating as the D-values were more than an order of magnitude shorter. During continuous-flow microwave heating, for PME in orange juice (pH 3.7), reference D_{60} was 12.4 s with a z-value of 10.2°C as compared to 155 s with z-value of 17.7°C obtained during conventional thermal treatment. For S. cerevisiae (ATCC 16664) in apple juice (pH 3.4), reference $D_{57.5}$ was 1.04 s with z-value of 7.7°C as compared to 16.0 s with z-value of 13.4°C and, for L. plantarum (ATCC 14917), reference $D_{57.5}$ was 13.3 s with z-value of 4.5°C as compared to 36.8 s with z-value of 15.9°C. The differential inactivation of PME between thermal and microwave heating was further

explored by evaluating the inactivation kinetics under batch-mode microwave heating conditions. Compared to conventional thermal inactivation, microwaves again showed to be more efficient. Non-thermal microwave effects were hypothesized to responsible for the differences in inactivation/destruction kinetics of the two heating modes.

Further investigations were carried to elucidate the existence of non-thermal effects of microwaves. Inactivation/destruction kinetics of PME in orange juice (pH 3.7) and *S. cerevisiae* (ATCC 14917) in apple juice (pH 3.4) were evaluated during microwave heating at low temperature in both continuous-flow and batch heating conditions. This study showed that the belief on existence of non-thermal microwave effects is, on the basis of this study, not appropriate since no significant inactivation/destruction was observed at non-elevated temperatures. In order to explain the differences between microwave and thermal heating modes, additional investigations were carried out with samples of various sizes subjected to progressively increasing temperatures in a batch mode. Results confirmed that there were additional effects associated with microwave heating which were both sample size and temperature dependent. The effect was, therefore, described as "*enhanced thermal effects* of microwave heating" than the traditionally used "*non-thermal* effects" which did not exist. The enhancement was characterized using a concept of mass normalized microwave heating time to effective thermal time ratio and a microwave enhancement ratio (MER) which was defined as the microwave to thermal inactivation/destruction ratio. These were quantified and microwave heating conditions which provide enhancement of thermal effects were elucidated.

RECOMMENDATIONS FOR FUTURE RESEARCH

Scope of the present study could be broadened by using established microwave kinetic data for industrial-scale microwave pasteurization. The research base could also be broadened by evaluating process and quality advantages of microwave heating with higher microwave power and heating volumes on a pilot scale. Numerical modeling of continuous-flow microwave heating could also be studied for easy scale-up considerations. The current research could be extended to other liquid foods such as milk and other fruit juices. The storage stability and retention of quality attributes such as flavors, colors and other organoleptic qualities would be an impetus aspect in order to demonstrate the commercial feasibility of the process.

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APPENDIX A

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C     FORTRAN PROGRAM TO CALCULATE EFFECTIVENESS OF CUT AND CDT
C     LINEAR APPROXIMATION FOR TEMPERATURE
C     TREF= REFERENCE TEMPERATURE
C     N= NUMBER OF TIME-TEMPERATURE DATA
C     M=NUMBER OF DATA POINTS AT EACH TIME INTERVAL
C     HTT= TOTAL HEATING TIME
C
      IMPLICIT REAL*8(A-H, O-Z)
      DOUBLE PRECISION TIME(1000),TEMP(1000),TIMEI(1000),TEMPI(1000)
      INTEGER I,J,K,N,M
C
      OPEN(5,FILE='TEST1.PRN')
      OPEN(6,FILE='TEST1.OUT')
      READ(5,*)TREF,Z,N,M,HTT
      DO 10 I=1,N
10    READ(5,*)TIME(I),TEMP(I)
C
      ALET=0.0D0
C
      DO 20 I=1,N-1
      J=I+1
      DELT=(TIME(J)-TIME(I))/M
      ALET=ALET+(10**((TEMP(I)-TREF)/Z))*DELT
C
      IF(M.LE.1) GO TO 20
      DO 30 K=1,M-1
      FAC=K
      TIMEI(K)=TIME(I)+DELT*FAC
      IF(TIMEI(K).GT.HTT) GO TO 50
      CALL AINT(TIME(I),TIME(J),TEMP(I),TEMP(J),TIMEI(K),TEMPI(K))
      ALET=ALET+(10**((TEMPI(K)-TREF)/Z))*DELT
30    CONTINUE
20    CONTINUE
      ALET=ALET+(10**((TEMP(N)-TREF)/Z))*DELT
50    EFF=(ALET/HTT)*100.0D0
      WRITE(*,*)EFF
      STOP
      END
C
      SUBROUTINE AINT(TIME1,TIME2,TEMP1,TEMP2,TIMEX,TEMPX)
      IMPLICIT REAL*8(A-H,O-Z)
C
      SLOPE=(TEMP2-TEMP1)/(TIME2-TIME1)
      TEMPX=TEMP1+SLOPE*(TIMEX-TIME1)
      RETURN
      END
```